

ABSTRACT OF THESIS

Name of Candidate VICTORIA BAGSHAW.
Address
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Title of Thesis Changes in ultrastructure during the development of callus cells.
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This thesis records a preliminary survey of the ultrastructure of Jerusalem artichoke tuber cells. The cells have been examined in freshly excised storage tissue from dormant mature tubers and under conditions of culture, on a nutrient medium, which cause them to grow and divide rapidly. This system, although not ideal for ultrastructural investigation because of the size and vacuolation of the cells has the particular advantage that the proportion of cells which are induced to divide at the first cell division do so synchronously. In nearly every respect the ultrastructure of the growing cells differed from its quiescent, non-dividing, counterpart. The organelles described include the nucleus and nucleolus, plastids, mitochondria, crystal-containing bodies, lipid bodies, endoplasmic reticulum, ribosomes, microtubules, dictyosomes, plasmalemma, paramural bodies, tonoplast and vacuole.

One of the most interesting problems in this investigation was how the movement of the nucleus, prior to division was organised. The observations made on the 'nuclear extensions' (structures apparently undescribed previously), their associated microtubules and endoplasmic reticulum suggest a) that the function of the microtubules might be to orientate the nucleus prior to cell division; and b) that the microtubules themselves may be controlled through the endoplasmic reticulum and the nuclear extensions by the nucleus. In addition the nuclear extensions and microtubules may provide support for the developing transvacuolar cytoplasmic strands.

The sequence of events during mitosis appeared to follow the usual pattern.

The most prominent organelles in dormant Jerusalem artichoke tuber tissue were the plastids. In the freshly excised tissue they were frequently found in tight clusters near the nucleus. These appeared to disperse during early culture. The plastids were found to contain two distinct and apparently unconnected membranous systems. These have been called the central system and the peripheral system. They remained distinct with culture but under certain conditions the central system formed grana and stroma lamellae. These were found only where the cultures turned green.

A variety of mitochondrial profiles were found in freshly excised tuber cells but cultured explants contained an even wider range of forms. These included complex bell shapes, long cylindrical rods, branched structures and plates. The complex bell-shaped mitochondria, and associated forms, are probably derived from simple spheres and rods.

CHANGES IN ULTRASTRUCTURE
DURING THE DEVELOPMENT
OF CALLUS CELLS

by

Victoria Bagshaw

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CONTENTS

| | |
|---------------------------------------|--------|
| INTRODUCTION | Page 1 |
| EXPERIMENTAL MATERIAL | 7 |
| EXPERIMENTAL METHODS | 8 |
| I CULTURE PROCEDURES | 8 |
| A. Preparation of Explants | 9 |
| B. Culture Media | 10 |
| C. Cultural Techniques and Conditions | 11 |
| II ELECTRON MICROSCOPY | 13 |
| A. Preparation | 13 |
| B. Fixation | 16 |
| C. Dehydration | 18 |
| D. Infiltration and Embedding | 18 |
| E. Sectioning and Staining | 20 |
| III LIGHT MICROSCOPY | 21 |
| A. Fixation | 21 |
| B. Dehydration | 22 |
| C. Infiltration and Embedding | 22 |
| D. Sectioning and Staining | 23 |
| IV HISTOCHEMICAL TECHNIQUES | 24 |
| A. Localisation of Iron | 25 |
| B. Localisation of Protein | 26 |
| C. Localisation of Starch | 26 |
| D. Localisation of Acid Phosphatase | 27 |
| E. Localisation of Lipid. | 28 |

EXPERIMENTAL RESULTS

Page 29

SECTION I UNCULTURED MATERIAL

29

A. Tubers

1 Freshly harvested mature tubers

30

a) Nucleus and Nucleolus

31

b) Plastids

32

c) Mitochondria

40

d) Crystal-containing bodies

44

e) Lipid bodies

46

f) Endoplasmic reticulum and ribosomes

46

g) Microtubules

47

h) Dictyosomes

47

i) Plasmalemma and associated structures

49

j) Tonoplast and vacuole

50

2 Stored tubers

52

3 Immature tubers

52

B. Other Organs

54

1 Green fully expanded leaf

54

2 Etiolated leaf

54

3 Etiolated young stem

55

4 Adventitious root

57

SECTION II CULTURED TUBER CELLS

58

A Changes Occurring during Short Term Culture

58

1. The Rind

60

2 Changes occurring before phragmosome formation

62

3 The Phragmosome (Early prophase)

65

4 Mitosis (Late prophase-telophase)

69

5 Cytokinesis

70

| | |
|--|---------|
| B Changes occurring during Long Term Culture | Page 72 |
| 1 Plastids | 73 |
| a) Cultures which did not turn green | 74 |
| b) Cultures which did turn green | 75 |
| 2 Mitochondria | 76 |
| 3 Other organelles | 79 |
| a) Crystal containing bodies | 79 |
| b) Lipid bodies | 80 |
| c) Endoplasmic reticulum | 80 |
| d) Dictyosomes | 80 |
| DISCUSSION | 82 |
| SUMMARY | 104 |
| BIBLIOGRAPHY | 107 |
| APPENDIX I Quantitative data on mitochondria | 122 |
| APPENDIX II Publications of results presented in this thesis | 123 |
| PLATES 1 - 72 | |
| Abbreviations in legends. | 124 |
| Abbreviations in plates | 125 |

INTRODUCTION

The purpose of this investigation was an examination of the ultrastructure of cells of the Jerusalem artichoke (Helianthus tuberosus) tuber and the changes which occur in them during culture. When explants isolated from these tubers are cultured aseptically in contact with a nutrient medium containing sugar, mineral salts, coconut milk and 2-4 dichlorophenoxyacetic acid, cell division is initiated in a proportion of the constituent cells and a callus culture is established (Yeoman, Dyer and Robertson, 1968). The first cell division occurs after a short lag phase and takes place almost exclusively in cells forming the outer 6 or 7 layers of cells in the cylindrical explant. It is the observations made on these layers of cells which are reported in this thesis. The first two cell divisions are synchronous (Yeoman, Evans and Naik, 1966; Yeoman and Evans, 1967), but this synchrony is lost with continuing cell division. During this period the original cellular pattern is partially obliterated except in the inactive central core. After 7 or 8 days differentiation occurs: tracheids appear in large numbers and distinctive nodules, which resemble meristems, are formed. They do not develop further under the cultural conditions used in this laboratory.

It was anticipated that an examination of the ultrastructure of the tuber cells would reveal contrasts between mature, quiescent storage cells and similar cells which had been stimulated to divide. Other ultrastructural studies on tissue cultures have been made on parenchyma storage cells of the carrot root (Israel and Steward, 1966)

and on batch-propagated suspensions of cells from sycamore (Sutton-Jones and Street, 1968).

It is essential in a study of this kind to obtain a thorough understanding of the state of the cells in the tissue at the beginning of the developmental sequence i.e. before culture is commenced. Therefore the starting point of the investigation was a survey of the ultrastructure of the cells immediately after excision from the tuber. Details of the arrangement and structure of all the organelles were recorded and compared with those of organelles in other quiescent cells such as carrot storage cells (Israel and Steward, 1966).

At the time of excision from the tuber the explant is composed of large vacuolated parenchymatous cells with uniform DNA (Partanen, 1959; Adamson, 1962; Naik, 1965; Mitchell, 1967), RNA and protein (Mitchell, 1968; 1969) contents. It has been shown that during the lag phase which precedes the first synchronous cell division DNA (Mitchell, 1967; Evans, 1967), RNA (Evans, 1967; Fraser, 1968), and protein (Evans, 1967; Mitchell, 1968) are synthesized and accumulated. The evidence presented by these authors strongly suggests that there is a high degree of synchrony in the metabolism of the cells which are going to divide. Therefore it was considered probable that other changes such as those in the ultrastructure of the cells, occurring during the lag phase would also take place synchronously.

The observations on the cultured material fell automatically into two sections: firstly, the observations made during short term culture on an initially uniform population of cells, some of which were induced to divide synchronously, and secondly, the observations made

made during long term culture, i.e. after the first two synchronous cell divisions, when the synchrony of the tissue was lost and the population of cells was no longer uniform.

It was hoped that the observations on the ultrastructure of cells in the lag phase could be related to their changing metabolism. It is inevitable that some of the changes occurring in the cells during the lag phase and the first division will not be typical of a normal division cycle but will be associated with the wound reaction of the excised tissue and the induction of a higher metabolic rate.

As stated above during the lag phase, RNA (Evans, 1967; Fraser, 1968), and protein (Evans, 1967; Mitchell, 1968) are synthesized and accumulate. It was therefore expected that the ribosome complement of the tuber cells might show a corresponding change. Nicholson and Flamm (1965) isolated ribosomes from cultured tobacco cells and they found that the ribosomes bound to the endoplasmic reticulum increased in young cultures and were active in protein synthesis. On the other hand, Israel and Steward (1966) and Sutton-Jones and Street (1968) reported increases in free ribosomes in young actively growing cultures of carrot and sycamore tissues respectively.

One physiological change which occurs during the lag phase is an increase in the rate of gaseous exchange of the artichoke tuber cells (Evans, 1967). The mitochondria in these cells were therefore examined to determine whether this change in the rate of gaseous exchange was associated with mitochondrial changes.

In the Arum spadix, Simon and Chapman (1961) found that a rise in 'respiration rate' was paralleled by an increase in the activity of the isolated mitochondria and by an increase in the structural complexity of

the mitochondria. Cherry (1963) found that increased mitochondrial activity in Arachis cotyledons was accompanied by a visible increase in the number of cristae in the extracted mitochondria.

After the lag phase, the length of which varies with the length of storage of the tubers (Evans, 1967), the first cell division occurs.

From light microscope observations it has been described that when highly vacuolated cells divide the nucleus is situated in the centre of a transvacuolar strand called a phragmosome (Bailey, 1920; Sinnot and Båoch, 1940, 1941; Steward, Mapes and Smith, 1958; Jones, Hilbebrandt, Riker and Wu, 1960). A similar condition had been observed in the tuber cells (Robertson, 1966). This change in the arrangement of the cytoplasm in the cells must involve cytoplasmic streaming. Microtubules (Ledbetter and Porter, 1963, 1964; Kane, 1962) and microfibrils (Nagai and Rebhun, 1966; Cloney, 1966; O'Brien and Thimann, 1966) have both been suggested as structures which may be involved in streaming and the tuber cells were examined in order to detect one or both of these structures. Microtubules have also been associated with other phases in the cell cycle and particularly with cell division. Pickett-Heaps and Northcote (1966b) reported that the position of the future new cell wall was indicated by a preprophase band of microtubules. In a later communication Burgess and Northcote (1967) suggested that the function of this band was to orientate the nucleus prior to division. The mitotic spindle is also composed of microtubules (e.g. Harris and Bajer, 1965) and microtubules can be found at the edge of developing cell plates during telophase (e.g. Pickett-Heaps and Northcote, 1966a, b, c).

Dictyosomes also play an important role in cell division. Whaley and Mollenhauer (1963) postulated that "the golgi apparatus produces

vesicles which fuse to form the cell plate, the golgi product thus contributing part of the substance of this structure and the golgi vesicle membranes contributing new plasma membrane". Since this postulate was put forward a large number of reports have been published on the role of the dictyosome during cell plate and cell wall formation. These reports include those of Frey-Wyssling, López-Sáez and Mühlethaler (1964), Esau and Gill (1965), Whaley, Dauwalder and Kephart (1966), Pickett-Heaps and Notthcote (1966a) and Pickett-Heaps (1967). It was therefore expected that the dictyosomes in the tuber tissue would show a response during the induction of cell division.

Observations made after the first two synchronous cell divisions were confined to changes apparently associated with a long culture period and not with cell division. The explants were cultured for up to 6 weeks and the plastids and mitochondria were accorded the greatest attention.

Recently a number of reports have been published on the ultra-structure of plastids in non-green tissues and some of these plastids have been shown to be highly complex. The plastids described include those from Jerusalem artichoke tubers (Gerola and Dassù, 1960), carrot storage tissue (Israel and Steward, 1967), bean roots (Newcomb, 1967), potato tuber buds (Marinos, 1967) and shoot apices of Bryophyllum and Kalanchoë (Gifford and Stewart, 1968). Work on plastids in tissue cultures includes that of Gerola and Dassù (1960) on artichoke tuber tissue, Laetsch and Stetler (1965) on tobacco tissue, Israel and Steward (1967) on carrot tissue and Sunderland and Wells (1968) on callus tissue of Oxalis dispar. The development of chloroplasts was studied in all of these tissue cultures. However Sutton-Jones and Street (1968) reported that only proplastids

and amyloplasts were observed in their cultures of sycamore cells. A variety of different cultural conditions were used in the present investigation of tuber cells to observe changes, if any, in the plastids.

Jones et al. (1960) reported that in aging tobacco cell cultures, observed with the light microscope, the round-oval mitochondria linked together in filiform aggregates a few days before the cells died. Israel and Steward (1966) found that the mitochondria in electron micrographs of older carrot cultures were elaborate and branched. No mention was made of the death of these cells. The shapes of mitochondria in the cultured tuber cells were observed.

Electron microscopic studies by themselves present a collection of micrographs of static phases and usually represent only a very small portion of tissue. In order to make these studies more valuable in the interpretation of a developmental pattern it is essential to use a well characterised system such as that of the artichoke tuber. It is hoped that the results presented in this thesis together with the histochemical and physiological studies which have been and are still being made in this department will lead to a greater understanding of the induction of cell division in tissue isolated from Jerusalem artichoke tubers.

EXPERIMENTAL MATERIAL.

The plant used throughout this investigation was the Jerusalem artichoke, Helianthus tuberosus L. cultivar Bunyard's Round. Clonal material was grown in the Royal Botanic Garden, Edinburgh and also in the garden of the Botany Department, Kings Buildings, Edinburgh. Tubers were planted in the soil in the open in March and the crop was harvested in November, by which time the aerial parts of the plant had almost completely died down. The harvested tubers were placed in polythene bags containing damp sand. These bags were covered with sand in large polythene bins and stored in a cold room at approximately 4°C. Under these conditions of storage the tubers were prevented from sprouting until May or June of the following year.

Most of the observations described in this thesis were made on pieces of tissue excised from freshly harvested tubers, from stored tubers and in cultured explants from these tubers. Further observations were made on pieces of fully expanded leaves from plants grown in the garden, on etiolated leaves, and shoots and adventitious roots from sprouting tubers stored in the dark, and on pieces of tissue from small immature tubers. These immature tubers were removed from plants on which the leaves were still fresh and green and presumably still photosynthetically active.

EXPERIMENTAL METHODS.

I CULTURE PROCEDURES.

At the start of this investigation explants were prepared in the light and cultured singly in the dark on a standard medium. (Yeoman, Dyer and Robertson, 1965). The explants were subsequently only exposed to light when examined and harvested. During this investigation improved methods for the culture of explants were developed in the Botany department. These improvements have been incorporated into the techniques used and are discussed here under i) short term culture (culture up to the end of the first synchronous cell division), and ii) long term culture (culture up to 6 weeks).

i) Short term culture.

The improvements for this period of culture relate to the early development of the callus and were designed to increase the synchrony and the proportion of cells dividing at the first division. The following improvements were made :-

a) The mass culture of explants (Yeoman, Evans and Naik, 1966; Yeoman and Evans, 1967 ; Fraser, Loening and Yeoman, 1967). Mass culture made it possible to set up a large number of explants simultaneously and increased both the synchrony and proportion of cells dividing at the first division.

b) The preparation and culture of explants under controlled light conditions. Light was found to have an inhibitory effect on the first cell division (Fraser, Loening and Yeoman, 1967). Therefore explants were prepared in low intensity green light and cultured in complete darkness.

9

In addition, some short term experiments were performed jointly with other members of the department, using a variety of techniques, with the intention of correlating the ultrastructure of the material with the results obtained in different investigations.

ii) Long term culture.

A high degree of synchrony and a large proportion of cells dividing at the first cell division are not so important for long term culture. Therefore techniques designed to promote these are not required. Also the improvements used for short term culture may be disadvantageous for long term culture. For the latter the culture of single explants on agar had the following advantages over mass culture in liquid:

- a) Sterile precautions were not required for harvesting.
- b) Contamination was restricted to individual cultures.
- c) A variety of cultural conditions could be conveniently employed on a small number of explants.

A. Preparation of Explants.

Cylindrical explants 2mm in diameter and 2.4 mm in length were prepared under sterile conditions according to the method described by Yeoman, Dyer and Robertson (1965).

All experiments were set up in a sterile room. Instruments and glassware were dry sterilised in an oven at about 300°F for at least 2 hours and the medium and the distilled water were sterilised in an autoclave at 15lb pressure for 20 minutes. Whole tubers were scrubbed in tap water, surface sterilised with sodium hypochlorite (2-3% v/v available chlorine) and then washed in sterile distilled

water. Cores of tissue, 2mm in diameter, were removed, with a stainless steel cannula, from a transverse slice, approximately 3 cm thick, from the central region of the tuber. The cores were then cut into 2.4 mm lengths with a special cutter.

The explants were prepared in fluorescent light (approx. 18fc.) or in low intensity green light.

B. Culture Media.

Three nutrient media were used in the experiments. These were a standard medium and two variants.

The standard medium was a solution of mineral salts and sugar, developed by Bonner and Addicott (1937) to which coconut milk and 2,4-dichloropheoxyacetic acid, (2,4 D) had been added (Yeoman, Dyer and Robertson, 1965). The medium had the following composition:-

| | |
|---|-----------|
| Coconut milk | 200 ml. |
| Sucrose | 40 g. |
| 2,4D | 0.22 mg. |
| $\text{Ca}(\text{NO}_3)_2$ | 236 mg. |
| KH_2PO_4 | 12 mg. |
| $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ | 36 mg. |
| KNO_3 | 81 mg. |
| KCl | 65 mg. |
| FeCl_3 | 2 mg. |
| Distilled water to | 1,000 ml. |

The final concentration of 2,4D was 10^{-6} M.

When a solid medium was required 1% Davis agar was added to the mixture.

The two variations of the standard medium were as follows:

- 1) a medium containing mineral salts, sucrose and 2,4 D (10^{-6} M) as in the standard medium, but no coconut milk.
 - 2) a medium containing mineral salts and sucrose as in the standard medium but a higher concentration of 2,4D (10^{-5} M) and no coconut milk.
- The standard medium was used for the majority of experiments reported in this thesis. The first variation of the standard medium was used to study the greening of cultures. The second variation was used in one short term experiment performed in conjunction with Fraser in this department.

C. Cultural Techniques and Conditions.

Four cultural techniques were employed in this research. For all the techniques used changes in anatomical structure, fresh weight and cell numbers were essentially the same. This electron microscopic investigation also showed similar results for the different techniques. The four techniques and the cultural conditions are outlined below :-

1) Stationary culture in bottles (Yeoman, Dyer and Robertson, 1965).

Individual explants were placed on their sides on 5ml or 10ml of standard medium or medium without coconut milk solidified with 1% agar, in screw top bottles.

Explants in initial experiments were cultured in the dark except when the material was harvested or examined. In later experiments some explants were cultured in continuous light (900fc.) whilst others which had been prepared in low intensity green light, were cultured in

complete darkness. Some of the explants cultured in the dark were later placed in continuous light. This method was used for some short term cultures and also most long term cultures.

2) Culture in roller tubes (Yeoman, Dyer and Robertson, 1965).

Single explants were impaled on a glass spike drawn out from the cut edge of a pyrex rod. Each rod was placed in a test tube containing 3 ml of standard medium and covered with an aluminium cap. The tubes were rotated in a roller tube apparatus and the explants were cultured in the dark except when the material was harvested or examined. Only one long term experiment was carried out using this method as it was not found to have any marked advantages over the agar method for obtaining suitable samples for electron microscopy.

3) Culture in conical flasks on a magnetic stirrer (Yeoman and Evans, 1967).

About 100 explants were grown in 15ml of standard medium in a 100ml conical flask containing a magnetic stirring bar. The explants were cultured in the dark except when harvested or examined. These conditions were used only for short term culture.

4) Culture in petri dishes on a reciprocating shaker. (Fraser, Loening and Yeoman, 1967).

About 80 explants were grown in 12ml of medium containing mineral salts, sucrose, $10^{-5}M$ 2,4D but no coconut milk in a petri dish (9cm in diameter.). The petri dishes were placed on a reciprocating shaker. One short term experiment was performed using this technique. The explants were prepared in low intensity green light and cultured in complete darkness.

All cultures were maintained at 25°C.

II ELECTRON MICROSCOPY.

The methods used in preparing samples for electron microscopy were basically those of Tulett (personal communication) who had carried out a preliminary investigation on artichoke tuber tissue.

A. Preparation.

It was anticipated that whole explants, especially when cultured for long periods would be too large for successful embedding. This section deals with the subdivision of the explant for fixation and embedding.

The choice of technique for subdividing explants depended on the following considerations:-

1) Nearly all the cells dividing occur in the peripheral regions of the cultured explant. These peripheral regions include the ends as well as the sides of the cylindrical explants. In liquid culture the cell divisions are distributed over the whole surface of the explant where as cell divisions in explants cultured on agar tended to be restricted to the surfaces not in contact with the agar medium.

2) Many of the cell divisions, especially the initial ones, are orientated to give a regular pattern of growth.

3) Degenerative changes occur on the outside of the explant. These involve not only the layer of cells cut during excision of the explant from the tuber but also underlying apparently undamaged cells. (Yeoman, Naik and Robertson, 1968).

It was essential therefore, to be able to relate what was seen in the electron microscope to the position and the orientation of the section in the whole explant. The three techniques described below for

subdividing explants were designed for this purpose. The first technique was used for cultures grown on agar; the second for explants grown on glass spikes in roller tubes, and the third method was finally used for all cultural techniques.

The subdivision of explants cultured on agar was carried out as follows: a minute red spot of safranin was painted on the upper surface of the explant before the explant was removed from the agar. The explant was then halved longitudinally but the top half only was kept and the half originally in contact with the agar was discarded. The top half was also often again cut longitudinally. Therefore the tissue embedded was approximately a quarter of a cylinder. After four weeks culture it had a radius of up to 2 mm and a length of up to 4 mm. Similar sized pieces of tissue were also obtained by longitudinally quartering explants grown on glass spikes in liquid culture.

The third technique was adopted primarily to reduce the size of the piece of tissue embedded. It exploited the fact that surfaces of tissue cut with a sharp razor blade before or after fixation could usually be distinguished from the outside of the whole explant when sections were examined in the electron microscope.

The technique consisted of cutting a thin disc of tissue off each end of the cylindrical explant. The discs were cut off either before or after fixation. The size of each disc was 2.4 mm in diameter and about 0.5 mm thick.

Satisfactory fixation and embedding was obtained at first with the fairly large pieces of explants prepared by the first two methods.

Later some difficulty was experienced in embedding material (see Section IID) and it was hoped to overcome this by embedding smaller pieces of tissue prepared by the third method. However, reducing the size of the tissue did not eliminate this problem. It was also found in sections from some of the thinnest discs prepared for electron microscopy that many cells had completely disorganised contents.

B. Fixation.

Fixation was usually carried out in glutaraldehyde followed by osmium tetroxide.

Early batches of glutaraldehyde, obtained from Koch-Light Laboratories Ltd., Colnbrook, were strongly acidic and were neutralised with sodium hydroxide before dilution with buffer. Later samples of glutaraldehyde, obtained from TAAB Laboratories, Reading, "as specially purified and stabilized for use as a fixative in electron microscopy", did not require neutralisation.

The procedure was to fix pieces of tissue in 6% glutaraldehyde in 0.1M phosphate buffer at pH 6.9. The fixation was started at room temperature for at least 2 hours and was continued overnight at 5°C. Fixation was carried out in complete darkness for tissue grown in complete darkness. The tissue often floated in the fixative at first but usually sunk overnight. Tissue which did not sink during this time was kept under vacuum until it sank. After this fixation the tissue was washed over a period of three hours in several changes of the phosphate buffer. The washing was to ensure complete removal of all excess glutaraldehyde, (Gunning, 1965.). Post fixation was then carried out for 1 hour in 1-2% unbuffered osmium tetroxide solution for 1-2 hours in 2 % unbuffered aqueous potassium permanganate. The washing and post fixation were usually carried out at room temperature. The glutaraldehyde and permanganate fixed material was very brittle when embedded and therefore was not used a great deal as thin sections could not be cut.

The fixation of artichoke tuber tissue with glutaraldehyde and osmium was found to be adequate though considerable damage was

observed in some cells, particularly common was the detachment of the tonoplast and the scattering of organelles in the vacuole. A great variation in the density of the ground substance was also found. The disorganisation of cell contents and variation in the density of the cytoplasm are thought to be the result of fixation and not the dehydration and embedding. The reason for this conclusion is that cells which were cut open immediately after fixation maintained normal cytoplasmic arrangement and structure. Therefore the contents of these cut cells were adequately fixed before the cutting and the dehydration and embedding did not alter the preservation.

Variations in the temperature during fixation and in the duration of fixation had no detectable effects on the quality of preservations. Neither the use of more dilute solutions of glutaraldehyde (1%) nor the addition of plasmolysing concentrations of mannitol to the fixative prevented detachment of the tonoplast. It is therefore concluded that the detachment is not caused osmotically. O'Brien (1967) has recently commented on the detachment of the tonoplast in preparations of Avena coleoptiles.

It is desirable to base ultrastructural results on a variety of fixatives as the detail of structure may vary with different fixatives. However the material used in this study did not fix satisfactorily with either potassium permanganate or osmium tetroxide alone (Tulett, personal communication). Although other workers have obtained successful preparations with these fixatives on storage tissue, e.g. Israel and Steward (1966) using tissue from the carrot root, or other highly vacuolate cells e.g. Esau and Gill (1965) using petioles of Beta. Acrolein (Tulett, personal communication) was found to give a similar quality of fixation to glutaraldehyde and osmium.

C. Dehydration.

Dehydration was carried out in ethanol.

The tissue was rinsed in 25% ethanol to remove excess osmium tetroxide or potassium permanganate, and dehydrated at room temperature in a 25%, 50%, 75%, 100%, ethanol series. The tissue remained in each solution for at least $\frac{1}{2}$ hour. The tissue was left in a fresh change of absolute alcohol overnight (and sometimes for several days) at 5°C. This overnight dehydration was found to be essential for the larger pieces of tissue. The dehydration process was completed at room temperature in two further changes of absolute alcohol.

The quality of preservation was found to be similar whether dehydration was performed in the cold or at room temperature.

D. Infiltration and Embedding.

The tissue was usually embedded in araldite. The araldite mixture used consisted of 30.3g araldite CY212, 23.4 g hardener HY964 and 1g DMP 30. The constituents were weighed in a polythene bottle, which had a polythene screw cap, and were shaken vigorously for a few minutes. The mixture was left at room temperature to allow all the air bubbles to come to the surface and then it was either used for infiltration and embedding or was stored in the deep freeze until required. After storage the bottle was allowed to warm up to room temperature for an hour in a desiccator before being opened for use.

Before infiltrating with araldite the tissue was left in a 1:1 absolute alcohol:propylene oxide mixture for $\frac{1}{2}$ hour and this was followed by two changes of propylene oxide each for 15 minutes. The tissue was then transferred to a 1:1 propylene oxide:araldite mixture which was stirred until the tissue sank. The mixture was left at room

temperature in a fume cupboard for about 24 hours until the propylene oxide had all evaporated. The tissue was next transferred to freshly made araldite mixture for a further 24 hours at room temperature.

The technique used for embedding enabled the tissue to be orientated on a flat surface. Sections could then be cut so that their orientation was known with reference to the whole explant from which the sample was taken. The tissue was embedded in gelatin capsules (No. 00). The capsules were filled with freshly made araldite mixture and were put over single pieces of tissue which had been orientated for sectioning in a drop of araldite on a sheet of polythene. The filled capsules were left at room temperature for about 1 hour and were then placed in an oven at 60°C for about 16 hours to harden the araldite. The blocks were peeled off the polythene sheet and trimmed and sectioned when cold.

Some difficulties were experienced whilst embedding several batches of tissue. On many occasions tissue floated when put into the propylene:araldite mixture even though it might not have floated in the fixative. When this occurred successful embedding was never obtained. The tissue floated in the embedding medium and remained spongy in the hardened block. The floating was not due to the presence of air bubbles on the surface of the explant. Variations in the size of the tissue, the rate of dehydration, and whether propylene^{oxide} was used or not did not overcome this problem.

Many of the sections observed were found to be badly torn along the cell wall. The severity was not associated with the size of the piece of tissue embedded nor with the length of infiltration time (up to 4 days) at room temperature in the araldite mixture, nor with the length of polymerizing time (up to 3 days) at 60°C.

Embedding in Vestopal (Ryter and Kellenberger, 1958) and in colloidin before araldite (Potts, 1966) were tried without success.

E. Sectioning and Staining.

Sections were cut with glass knives on a Ruxley ultramicrotome. The sections were floated on 10% acetone or distilled water and those which showed silver/gold to grey interference colours (600-800A°) were picked up with freshly cleaned, dry, uncoated, copper grids (400 or 300 mesh). The grids were rinsed with distilled water to remove any dirt which might have been picked up from the trough.

Some sections were examined without being stained. Some of these were treated on the grids for 30 - 60 minutes with 2% hydrogen peroxide to oxidise and remove bound osmium (André and Marinuzzi, 1965) and were then examined. A few of the sections treated with hydrogen peroxide were also treated with 10% HCl for one hour before being examined.

Sections were stained on the grids with uranyl acetate (saturated aqueous solution) for 60-90 minutes and lead citrate for 5-30 minutes or with lead alone (5-30 minutes) by the methods of Reynolds (1963) and Venable and Coggeshall (1966). Alternatively sections were stained with either potassium permanganate or barium permanganate (20-30 minutes) by the method of Lawn (1960).

All the staining was carried out in closed petri dishes and the grids were floated on drops of stain on clean pieces of polythene. To prevent precipitates of stain on the section the following precautions were taken. After staining in uranyl acetate the grid was thoroughly rinsed with distilled water before staining with lead. During lead

staining the CO_2 in the atmosphere within the petri dish was reduced by enclosing a few sodium hydroxide pellets or filter paper soaked in a 1.0 N solution of sodium hydroxide. After lead staining the grid was rinsed with 0.02N sodium hydroxide solution to remove the excess lead solution. The grid was then rinsed with distilled water and dried by touching the edge of the grid with filter paper.

Sections were examined with an AEI EM6 electron microscope and electron micrographs were taken, at either 50 or 75 kV using a 50 mp objective aperture, on Ilford special lantern contrasty plates. The plates were developed in either ID 19 or microphen.

III LIGHT MICROSCOPY.

General anatomical observations on cell size and arrangement at the light microscope level were made on sections cut from the same araldite blocks as sections for electron microscopy. In addition a variety of fixatives were used in an attempt to make observations on plastids and mitochondria at the light microscope level. The fixed material was embedded in paraffin wax or 2-hydroxypropylmethacrylate (HPMA). Sections of tissue embedded in araldite and tissue embedded in wax were also used for histochemical tests.

A Fixation.

Five fixation procedures were used and are described below. In each case whole explants were fixed.

- 1) Explants were fixed (as for electron microscopy) in 6% glutaraldehyde in 0.1 M phosphate at pH 6.9. Fixation was started at room temperature for at least 2 hours and was continued overnight at 5°C. After fixation

the tissue was rinsed in at least two changes of phosphate buffer. This fixative was frequently used and provided good results for tissue embedded in wax.

- 2) Explants were fixed at room temperature in 6% acrolein in cacodylate buffer at pH 7.3 for 1 hour. This fixative gave very satisfactory results for tissue embedded in HPMA.
- 3) Explants were fixed in acrolein as above followed by 8 days post fixation in 3% potassium dichromate. This fixation procedure did not yield satisfactory results.
- 4) Explants were fixed in Regauld fixative for 5 days followed by 8 days post fixation in 3% potassium dichromate (Py, 1932) but no satisfactory results were obtained.
- 5) Explants were fixed in Zirkle's fluid for 48 hours at room temperature (O'Brien, 1951). Glutaraldehyde was found to give more satisfactory results than this fixative.

B. Dehydration.

Two dehydration procedures were used:-

- 1) Dehydration was carried out in ethanol and tertiary butanol according to Johansen (1940) before embedding tissue in paraffin wax.
- 2) Dehydration was carried out in ethanol as for electron microscopy before embedding tissue in HPMA.

C. Infiltration and Embedding.

The two embedding materials used were paraffin wax, MP 56°C, and 2-hydroxypropylmethacrylate (HPMA).

The tissue to be embedded in paraffin wax was placed in a 1:1 paraffin oil:tertiary butanol mixture for one hour. Infiltration with

wax was then commenced. A little melted wax was poured into a sample tube and allowed to solidify. The pieces of tissue were then placed on this wax and covered with more paraffin oil:tertiary butanol mixture. The samples were left in an oven at 60°C and one hour after they had sunk the liquid was poured off and replaced with pure melted wax. Two changes of melted wax were given within 6 hours and the tissue was left overnight in a further change. The tissue was then embedded in pure wax which was allowed to solidify at room temperature and then in cold water.

The HPMA embedding medium contained 95ml HPMA, 5ml carbowax (polyethylene glycol 200) and 0.15g of catalyst (2,2-azobis(2 methyl propionitrile)). The mixture was stored in a glass bottle at room temperature in the dark. After dehydration tissue to be embedded in HPMA was transferred to two changes of the plastic monomer for one hour each at room temperature and was then left overnight in another change of the mixture. The embedding was carried out in gelatin capsules (No. 00). The tissue was placed in the gelatin capsules which were then filled to the brim with monomer mixture and carefully capped to exclude as much air as possible. The HPMA was polymerised overnight in an oven at 60°C and then the gelatin capsule was removed with a razor blade leaving the block ready for trimming and sectioning.

D. Sectioning and Staining.

Araldite and HPMA embedded materials were sectioned with glass knives on a Huxley ultramicrotome. The sections (1-4µ thick) were lifted from the water in the knife trough by means of a fine wire loop and placed on a drop of water on a glass slide. The sections were then

dried onto the slide in an oven at 60°C and stained. The araldite sections were stained for 10 minutes with basic fuchsin which was heated until it steamed. The HPMA sections were stained with 1% acid fuchsin in distilled water for 2 minutes and 0.05% toluidine blue in benzoate buffer pH 4.4 for 2 minutes (Ashley and Feder, 1966). All the sections were mounted in Euparal when dry. From tissue embedded in wax, sections (8-10 μ thick) were cut on a Beck rotary microtome. The sections were picked off from the warm water bath with glass slides coated with glycerin albumen. The wax was removed from the sections with xylene and the sections were taken down in ethanol series as necessary before staining. They were dehydrated with the same series after staining and mounted in Euparal. The wax embedded sections were stained with Hedenhain's iron haematoxylin (Johansen, 1940) and by other techniques described in the following section.

IV HISTOCHEMICAL TECHNIQUES.

Observations on artichoke tuber tissue with the electron microscope indicated the presence of a variety of deposits which are identified in the literature as storage materials. Histochemical staining techniques were then used to study the distribution of iron, protein, starch and lipid at the light microscope level.

Previous work carried out in this department, (Robertson, 1966), indicated the presence of acid phosphatase in peripheral layers of cultured explants. The distribution of acid phosphatase was studied at the electron microscope level in this investigation.

A. Localisation of Iron.

The ferrocyanide test for iron (Gomori, 1952) was applied to 8 μ thick sections of stored tuber tissue which had been fixed in glutaraldehyde and embedded in wax. The wax sections were dewaxed in xylene and taken through an ethanol series to water. They were then placed in a freshly prepared filtered solution of 10% potassium ferrocyanide for 5 minutes, followed by 30 minutes in a 1:1 mixture of 10% potassium ferrocyanide and 10% analar hydrochloric acid. The sections were then washed in running tap water, dried and mounted in Euparal. The ferrocyanide test was also applied to araldite sections, 4 μ thick, of freshly harvested tuber tissue which had been fixed in glutaraldehyde and osmium and embedded in araldite. These araldite sections were treated for longer periods of time to allow for the slower penetrations of the solutions through the araldite. The sections were first treated for one hour with 2% hydrogen peroxide to oxidise and remove the bound osmium (André and Marinozzi, 1965). They were then placed in 10% potassium ferrocyanide solution for 15 minutes followed by 1 hour in a 1:1 mixture of 10% potassium ferrocyanide and 10% hydrochloric acid.

Controls for the test for iron consisted of i) sections treated for 15 minutes (paraffin wax) or 1 hour (araldite) with 10% hydrochloric acid before immersion in potassium ferrocyanide and ii) sections treated with distilled water instead of potassium ferrocyanide.

Slides were examined under bright field and phase contrast light conditions.

B. Localisation of Protein.

The mercuric bromophenol blue stain for protein (Mazia, Brewer and Alfert, 1953) was applied to 8 μ thick sections of stored tuber tissue which had been fixed in glutaraldehyde and embedded in wax. Attempts to use araldite sections (Newcomb, 1967) were frustrated by the complete loss of the sections, free or attached to microscope slides in the staining solutions.

Wax sections were placed in xylene to remove the wax, were rinsed in absolute alcohol and 95% alcohol and then were stained in 0.1% bromophenol blue in 10% mercuric chloride (in 95% ethanol) for one hour. The sections were rinsed in water and washed for 20 minutes in 0.5% acetic acid and for one minute in three changes of water. They were left to dry and then rinsed in xylene, mounted and examined with the light microscope.

C. Localisation of Starch.

Iodine solution and the periodic acid - Schiff technique were used to localise starch.

Hand cut sections of fresh green leaves, immature tubers and etiolated stem apices were cut, and stained on a slide with a few drops of iodine. The sections were examined with the light microscope.

The periodic acid - Schiff test for insoluble carbohydrates was carried out on 2 μ thick araldite sections of glutaraldehyde and osmium fixed tissue from freshly harvested mature tubers and from cultured explants. The sections were treated with 1% aqueous periodic acid for 2 hours with Schiff's reagent for one hour and with a

'sulphite rinse' (10 ml N HCl with 180 ml of distilled water) for one hour.

D. Localisation of Acid Phosphatase.

The Gomori reaction (Gomori ,1952) was performed on explants cultured in standard liquid medium. Whole explants were fixed at room temperature either for 5 hours in 5% glutaraldehyde or for 24 hours in 4% formaldehyde. Both fixatives were in 0.1 M sodium cacodylate buffer (pH 7.3). The explants were washed overnight in the sodium cacodylate buffer and the following day in four more changes (30 minutes each) of the same buffer. They were then halved longitudinally and transferred to the Gomori reaction mixture at 37°C for 5 - 10 minutes. This mixture had a pH 5.0 and was composed of 500 ml 0.05 M acetate buffer, 0.6 g $\text{Pb}(\text{NO}_3)_2$ (about 0.003M) and 50 ml 3% (about 0.1M) sodium glycerophosphate. Two control mixtures were also used. In the first of the controls, the glycerophosphate solution was replaced by distilled water and in the second 0.05 M sodium fluoride was used as an enzyme inhibitor. The explants were removed from the reaction mixtures and washed in the sodium cacodylate buffer for 10 - 30 minutes. They were then put into 1% acetic acid for 1 hour or overnight. They were washed in sodium cacodylate buffer for 10 - 30 minutes and fixed in 1% osmium tetroxide in sodium cacodylate for 1 hour at room temperature. The explants were then dehydrated in ethanol and embedded in araldite by the usual procedures for electron microscopy. Sections were cut and examined unstained.

E. Localisation of Lipid.

Sudan III was used as a test for lipids at the light microscope level. Handcut sections of etiolated stem apex and pieces of small stored tubers, which had been fixed in 6% glutaraldehyde overnight and immersed briefly in 50% alcohol were stained in a saturated solution of Sudan III in 70% alcohol for 5 - 15 minutes. The sections were briefly rinsed in 50% alcohol, washed and mounted in water and examined.

EXPERIMENTAL RESULTS.SECTION I UNCULTURED MATERIAL.

An essential prerequisite to an investigation on the changes in ultrastructure during the development of callus cells is an understanding of the structure of the tissue at the commencement of the developmental sequence. Accordingly the first part of this work was concerned with a detailed analysis of the ultrastructure and arrangement of organelles in the highly vacuolated storage parenchyma cells of the Jerusalem artichoke tuber. The ultrastructure of freshly harvested mature tuber cells was examined first. However these tubers were available only at the end of the growing season in November. It was therefore necessary to store tubers for use in experiments performed during the rest of the year. For this reason the investigation was extended to stored tuber cells in order to detect any changes in ultrastructure during storage. A few observations were also made on developing immature tubers in an attempt to record stages in the development on the organelles.

During the preliminary phase of the investigation great interest was stimulated by the arrangement and structure of plastids and this placed a heavy emphasis on these organelles. In order to understand the structure of the plastids more fully their ultrastructure in the tubers was compared to that of plastids in other organs of the Jerusalem artichoke plant. Accordingly green leaves, etiolated shoots and leaves and adventitious roots were examined.

A. Tubers.

i) Freshly harvested mature tubers.

Most of the observations on uncultured material were made on freshly harvested mature tubers. In most respects, the structure of stored and immature tubers was similar. Where appropriate, observations on stored and immature tubers are included in this section.

The explants were removed from tissue which consisted of fairly uniform parenchymatous cells (Plate 1A). Occasionally vessel elements or tracheids and secretory canal cells were also included. Only the structure of the parenchyma cells will be described here.

The parenchyma cells were up to 100 μ long and 50 μ wide and were highly vacuolated. As well as a large central vacuole (V) (Plates 1B & C) groups of small vacuoles were often present in the cytoplasm, particularly in the corners of the cells (Plate 2A). The cells had a thin layer (frequently less than 0.25 μ thick) of peripheral cytoplasm (Plates 1B & C) in which all the organelles including the nucleus (N) were embedded. The density of the cytoplasm in the cells, even adjacent ones on the same section was often found to vary considerably. At one extreme every membrane, ribosome etc, could be distinguished clearly against an electron transparent background whereas at the other extreme the cytoplasm was almost opaque and the position of the membranes only showed up in negative contrast. These differences in density were also observed in cultured material (Plate 2B). Presumably the dense appearance of some cells reflects differences in the cells before fixation. The contents of a few cells appeared very disorganised. (Plate 2C).

The different organelles found in these cells will now be

described.

a) Nucleus and Nucleolus.

The nucleus was always found close to the wall in a peripheral layer of cytoplasm. It was commonly disc shaped (Plates 3A & B) but was sometimes deeply lobed (Plates 4 & 5). In the latter case the spaces between the nuclear lobes were filled with cytoplasm and sometimes contained plastids (Plate 5), mitochondria and other cell organelles.

The nuclear envelope consisted of the usual two membranes and was perforated by nuclear pores. Ribosomes were sometimes detected on the outer membrane and this membrane was very occasionally seen to connect with the endoplasmic reticulum. No connections with mitochondria or plastids were observed, despite their close proximity to the membrane.

In some, but not all, of the nuclei dense aggregates of chromatin were found (Plates 3B & 4). The aggregations of chromatin varied in size and O'Brien (1967) reported that this feature is characteristic of a highly differentiated cell type. The aggregates were dispersed throughout the entire volume of the nucleus without the particular relationship to the envelope that is found in many differentiated animal cells (Porter and Bonneville, 1963).

The Jerusalem artichoke is an allohexaploid and up to six nucleoli have been seen in one nucleus. Each nucleolus (Nu) was usually rounded in outline and consisted of a central fibrillar region (pars amorpha)(F) , and the peripheral granular region (nucleolonema)(G)(Plate 4). This region has been called ribosomal (Hyde, 1966) because particles similar to cytoplasmic ribosomes have been prepared from isolated nucleoli. The granules, in this investigation, were found to be about 18mpu in diameter which was slightly smaller than the cytoplasmic ribosomes (20mpu). The elements in the two nucleolar regions were packed closely together

(Plates 5 & 7A)but in some sections less dense areas were seen associated with either region. The less dense areas appeared to be of two different types. The first type were more centrally placed and were surrounded by granular material. They also contained granules similar to the surrounding ones and there was some suggestion of fibrillar material in them (Plate 6). These less dense areas were only occasionally found in uncultured tissue but similar ones were commonly found in the dividing region of cultured tissue. The second type of less dense areas were smaller and found near the periphery of the nucleus. They were surrounded by fibrillar material similar in appearance to the extensions of heterochromatin (H) (see arrows in Plate 6) which were seen interrupting the circular outline of the nucleolus (Plate 7A). Hyde (1966) reported that the heterochromatin extensions and the smaller less dense areas containing similar material were connected.

Also partially embedded in the surface of the nucleolus were bodies (K) similar to karyosomes which have been described in peas by Sankaranarayanan and Hyde (1965). In the artichoke tuber they were characterised by fibrillar material which was usually less electron dense than the granular and fibrillar material of the nucleolus but more electron dense than the ground substance of the nucleus (Plate 7B) .

b) Plastids.

There was an enormous variety in the appearance of the plastids(P). The profiles varied in size, shape and in the presence or absence of the various features described below. Some of the simpler profiles (Plate 5) no doubt represent sections through less elaborate parts of extensive plastids, but much of the variation in the profiles probably

signifies a comparable variation in the structure of the plastids themselves. The variation appears to be continuous, however, and the plastids did not yet obviously fall into two or more distinct morphological classes. The plastids exhibited many of the phenomena associated with plastids in non-photosynthetic tissue e.g. potato tuber buds (Marinos, 1967) carrot storage tissue (Israel and Steward, 1967) and bean roots (Newcomb, 1967).

Plastids were found scattered in the thin regions of peripheral cytoplasm and also in clusters of up to approximately 100 in larger patches of cytoplasm. The clusters were most commonly found near to and even touching the nucleus, (Plates 3A, 4, 5 & 7C), but no connections between the plastids and nucleus were found. In the clusters each plastid appeared to consist of a large head (up to 7 μ long and 3 μ wide in profile) and a narrow tail (50 - 100 $m\mu$ in diameter). However connections between heads and tails were not seen in every section. The heads were arranged around the periphery of the clusters and the tails intertwined in the centre. This can be seen in Plates 3A, 5 and 7C but is most convincing in Plate 7C. Frequently sections showed two centres of intertwined tails in one cluster (Plate 3A) but serial sections of some of these clusters indicated that both tail regions were parts of one curved centre. However no clusters have been sectioned from one side of the central region to the other. The whole cluster was up to 20 μ across and the central region 3 μ .

The exact construction of the central region (Plates 8A, B & C) was not determined. Whether every tail was connected to a head or whether some of the tails were really small plastids is not known. No convincing branching of a tail was observed but close associations

with ER and a tubular structure (arrowed in Plates 8B & C) were sometimes found. Whether either of these were connected to the plastid envelope was not clear (Plates 8A,B & C). Occasionally short extensions of the outer membrane of the envelope were seen in the cytoplasm.

The variations in shape and size of the plastids can be seen in both clusters (Plates 3A,5,7C,8A,B & C) and scattered ones (Plates 2C,9A,B,C & 10A). There were apparently no consistent differences in structure between the clustered and the scattered plastids.

Narrow elongate profiles up to 10 μ long and only about 0.1 μ wide were often encountered in the thin layer of peripheral cytoplasm (Plate 9A). They are likely to have been sections through discs as one would not expect to cut longitudinally through rods so frequently. Wider profiles were found in larger patches of cytoplasm particularly near the nucleus. Circular, elongate, irregular and branched forms were all commonly found. A common characteristic of the plastids was the presence of one or more areas of 'encircled' cytoplasm (Plates 7C, 9B,C & 10A) like those described by Newcomb (1967) in bean roots. This cytoplasm was often less dense in appearance than the cytoplasm surrounding the plastids and was frequently found to communicate with it (Plate 9B). The encircled cytoplasm sometimes included a mitochondrion or other cytoplasmic organelle (Plate 10A). Plates 9B & C are serial sections of a plastid. In the first the encircled cytoplasm communicates with the surrounding cytoplasm but in the second it does not. It is not known if all regions of encircled cytoplasm had similar openings. The width of the openings was found to be 75 - 250 m μ which was considerably smaller than the longest length of the encircled cytoplasm profiles (up to 3 μ). Therefore most sections would not be expected to

cut through the opening even if one was always present. (The mitochondria in this tissue were similarly hollow (Plate 7C)).

The plastid envelope consisted of two membranes which were usually more electron dense than those of the mitochondria. Within the envelope the structure varied considerably. Embedded in the finely granular stroma were a number of components. These included osmiophilic droplets approximately 50 mμ in diameter, a complex of membranes and tubules, and electron dense granules and impenetrable masses. No starch bodies were found in plastids from freshly harvested and stored mature tubers. Fine fibrils were sometimes seen in clear areas, in the particularly around clusters of phytoferritin (arrows in Plate 10B). The fibrils were similar to those found to contain DNA by, for example Swift (1965), but no attempt was made to characterise the fibrils in the tuber plastids. Structures positively identifiable as ribosomes were not observed in plastids from uncultured material.

The complex of membranes and tubules comprised the following: a central system, an apparently independent peripheral system and distinct tubules.

The central system usually occupied central regions (hence the name) of the plastid and exhibited very variable morphology. It was delimited by a single membrane and its contents were typically more electron dense than the matrix of the plastid. The central system(C) sometimes consisted of a simple sac with or without more or less discrete channels or holes in it. (Plates 7C, 10A & 11A). Connected to this sac there was often a system of interconnecting cisternae and tubules of very irregular diameter (Plates 10A & 11A). In the most dissected regions these were similar in appearance to a crystalline

prolamellar body which had been exposed to a short period of light (Plate 11B) (e.g. Fig 15 in Gunning, 1965). Both sacs and inter-connecting tubules could be found separately and intermediate conditions were also found. Wisps of material apparently continuous with the rest of the central system were present, trailing off into very fine strands (arrowed in Plates 10A & 11C). Such strands were particularly conspicuous lying longitudinally in the 'tails' of clustered plastids (Plate 8A). The central region was up to $3\ \mu$ across and in the more dissected regions the tubules were 20 μ m or more in diameter. The simple sac corresponds to the corpo opaco of Gerola and Dassù (1960) who have also investigated the structure of plastids of artichoke tuber tissue. In the plastids of bean roots, Newcomb (1967) has described similar sacs containing material, sometimes crystalline, identified as protein on the basis of the staining reaction with mercuric bromophenol blue at the level of the light microscope. He also described a tubular complex continuous in places with the sacs and similar to, though more regular than, the more dissected parts of the central system in artichoke tuber. Israel and Steward (1967) have described a 'prethylakoidal body' in carrot tissue which resembles the corpo opaco, and they attribute the high electron density of this unit to a high concentration of protein. When sections of stored artichoke tubers were stained with mercuric bromophenol, small regions within the highly staining plastids were deeply stained (Plate 11D). These regions may have been the corpi opaci.

The peripheral system usually lay between the central system and the plastid envelope and some of the membranes of this system were seen to be continuous with the inner membrane of the plastid envelope.

(see arrows Plate 8A). The peripheral system (PS) consisted of irregular tubules and cisternae, 20 - 100 μ m in diameter, the contents of which were less electron dense than those of the central system (Plates 9A,B,C,10A,B & 11B). In some plastids there was a tendency for the flattened cisternae to be parallel to the surface of the plastid (Plates 9A & 10A). Israel and Steward (1967) have reported a distinctive peripheral system formed by invagination from the inner of the limiting membranes in the plastids of cultured carrot storage tissue. Similar invaginations have been reported in other plastids (e.g. Newcomb, 1967).

The central system and the peripheral system may have been continuous but in this investigation there was no unequivocal evidence of such connections although the two systems were often closely associated.

The third system consisted of unbranched tubules like those of the bean root plastids (Newcomb, 1967). The tubules (T) (Plates 9B,9C & 10B) were narrower and considerably more uniform in diameter (15 - 22 μ m) than those of the central and peripheral membrane systems. The tubules tended to lie with their long axes normal to the surface of the plastid. The tubules often ran between the envelope and the membranes of the other two systems but the nature of the connections, if any was not determined.

Electron dense material similar to that usually identified as phytoferritin (Pf) (e.g. Marinos, 1967; Robards and Humpherson, 1967) was found in the matrix. Granules, approximately 5 - 6 μ m in diameter, were found both widely scattered throughout all the matrix (Plate 12A) and in clusters which were characteristically present in the holes in the sacs of the central system or were surrounded by the cisternae of this system.

(Plates 11A & B). The clusters exhibited a variety of profiles including loosely packed granules (Plates 10B & 11A), dense impenetrable masses (Plates 8C & 10A) and crystals (Plate 12A & 13A). The dense impenetrable masses and crystals were often surrounded by scattered granules. Many of the observations on the electron dense material including results on the characterisation of the structure of the crystals were made on sections of immature tubers. A greater number of crystals were detected in the plastids of these tubers which made them particularly suitable for this part of the investigation. The ferrocyanide test for iron was carried out on sections of stored material as no freshly dug tubers were available at the time.

The relevant observations on this electron dense material were: a) The electron density was intrinsic. All forms were found in material fixed with glutaraldehyde without post-fixation in osmium and examined without staining with heavy metals (Tulett, personal communication). They were also visible when sections of material fixed with glutaraldehyde and osmium were treated with hydrogen peroxide to remove bound osmium and were then examined unstained (Plates 12A & B). b) Blue granules, presumably of ferric ferrocyanide were found when sections given the ferrocyanide test for iron were examined in the light microscope. Examination of such wax or araldite sections alternately in bright field and phase contrast conditions, suggested that all the blue granules were located within organelles, which were probably plastids (Plates 12C & D). The dense material in the electron micrographs were similarly confined to plastids, in itself a characteristic of phytoferritin (Hyde, Hodge, Kahn and Birnstiel, 1963). c) The blue granules did not form in the ferrocyanide test on sections pretreated

with 10% hydrochloric acid and the dense material was not visible in the electron microscope when sections were similarly pretreated with hydrochloric acid. d) The crystals had the same form and period as those illustrated by Hyde et. al.(1963) who extracted phytoferritin from pea material. A characteristic appearance of the crystals was that of alternating lines and rows of dots and the period was 9 - 12 μ . The lines and rows in this material were always straight. In other material, including sycamore (Catesson,1966), Beta (Cronshaw,Hoefert and Esau, 1966),Willow (Robards and Robinson, 1968), and pea root nodules (Dixon, personal communication.) they are characteristically curved. e) The individual granules in the crystals varied considerably in their electron density. This variation is characteristic of animal ferritin where it presumably reflects differences in the iron content of the granules. (Haggis,1965). f) The granules both isolated and in crystals (Plate 13A) showed the classical appearance of ferritin in underfocused micrographs, of usually 4 or 5 subparticles arranged at the corners of a square or in various other geometrical patterns. Such patterns, which are characteristic of ferritin and of phytoferritin (Hyde et al.,1963; Catesson,1966; Robards and Robinson,1968),were originally interpreted in terms of subparticles arranged at the vertices of for example an octahedron (e.g. Muir,1960.) but have more recently (Haggis,1965.) been interpreted as artefacts of underfocusing.

It seems reasonable to infer that the crystals and isolated granules were of phytoferritin, but less certain that the dense masses were not penetrated by the electron beam. A particle of ferritin, or phytoferritin consists of a dense core, about 5 μ in diameter, of a compound of iron in a shell of protein and is about 10 μ across.

This approximates to the spacing in the crystal. It is difficult, therefore, to imagine how ferritin could be packed more closely to give completely impenetrable masses. The masses may contain iron but the evidence presented under a), b), and c), above does not even prove this. However the close association of granules with the masses and the similar position of the masses to the granules and crystals suggests that masses are related to phytoferritin. In this thesis the name phytoferritin is used to include the impenetrable masses but it is realised that the nature of these masses cannot be finally determined until they have been isolated and characterised.

c) Mitochondria.

Mitochondria (M) were found scattered singly and in small groups throughout the cytoplasm. They showed a variety of profiles the simplest of which was circular with a diameter of up to 1.5 μ (Plates 2A & 10A). This may represent a section through a sphere or a cylindrical rod. The mitochondrial envelope consisted of the usual two membranes and after lead staining the inner one was often more electron dense than the outer. Cristae extended into a matrix and connections between cristae and the inner membrane of the envelope were sometimes seen. If the cristae in Plates 13B & 13C are compared it can be seen that those in Plate 13C appear to be inflated. Such variations in the appearance of the cristae were common between different cells but were not seen in any one cell. Whether they reflected differences between the cells before fixation or were the result of a fixation artefact is unknown. Similar differences have been found in germinating bean cotyledons by ["]Opik (1965). Some of the

cristae were long and irregularly arranged and in some of the mitochondria they formed a ring (Plate 13B). Sitte (1963) reported similar ring shaped structures in Elodea mitochondria, after osmium tetroxide fixation and to a lesser extent after potassium permanganate. Whether the ring shaped structures were fixation artefacts was not determined in this investigation.

The mitochondrial matrix was more electron dense than the cytoplasm. Electron dense granules (DG) were sometimes found in the matrix (Plate 14A). Peachey (1964) associated similar granules in animal mitochondria with the accumulation of divalent cations and he suggested that they regulated the internal ionic environment of the mitochondria. Lehninger (1964) also reported that similar granules were sites at which calcium salts were deposited.

Clear areas were detected in the matrix in some mitochondria. In a few of these areas fine electron dense fibrils were detected. These are arrowed in Plate 14A. Similar fibrils were reported in chick embryo mitochondria by Nass and Nass (1963) and they showed that the fibrils contained DNA. No attempt was made in this investigation to characterise the fibrils in the tuber mitochondria.

The presence of particles containing RNA and resembling ribosomes in animal mitochondria were reported by André and Marinozzi (1965). Granules similar to ribosomes were detected in the matrix of mitochondria in the tuber cells (Plate 14B) but whether they contained RNA or not was not determined. Sections treated with RNase were extremely electron dense.

Occasionally very electron dense circular bodies were seen in the mitochondria. These bodies appeared to consist of a whorl of membranes. (Plate 14B). Perry and Waddington (1966) reported that:

"In preparations fixed in glutaraldehyde it is relatively common to find myelin-like bodies associated with mitochondria" in the cement gland in Xenopus. The significance of these bodies is not known.

Oval profiles were common and there were a few elongate profiles (Plates 10A & 13B) indicating that at least some of the mitochondria may have been rod like. No branched structures were observed. Occasionally U-shaped profiles were seen (Plate 14C). In these the lateral arms were club shaped in profile and they incorporated the normal mitochondrial structure. The tapering ends of the two clubs were connected through a narrow bridge. It may be suggested that this profile represented a longitudinal section through a cup. Outlines in the form of a ring (Plates 13C,D & 14A) approximately 1.5μ in diameter were frequent. These were presumably derived from a transverse section through the sides of the cup (at the level $a_1 - a_2$ in figure 1.)

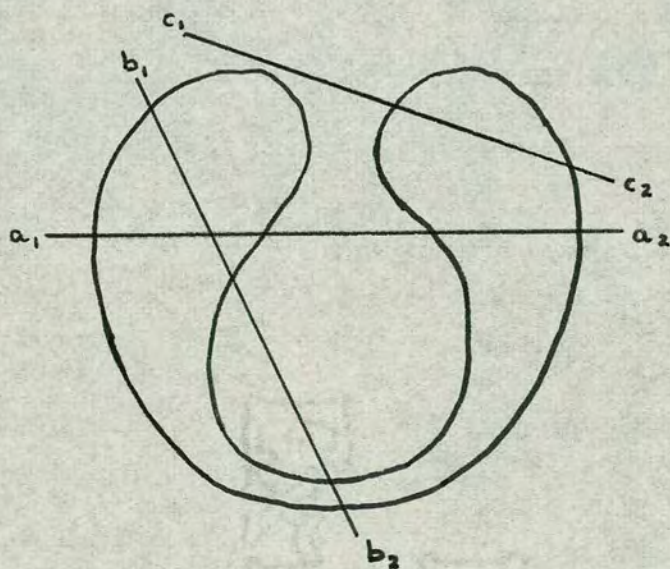


FIGURE 1. Cup shaped mitochondrion.

Plate 14A shows a mitochondrial arc, the edges of which are connected by a thin loop. This structure probably represents a section through the level of $b_1 - b_2$ in figure 1. Plate 14D probably represents a section through the level $c_1 - c_2$ in figure 1. Plate 14E may be interpreted in terms of two cups in the same structure at right angles to each other. However these two-cup structures were rare.

TABLE 1 . MITOCHONDRIAL COUNTS FROM SECTIONS OF
UNCULTURED TUBER TISSUE.

| <u>PROFILE.</u> | <u>SOLID CIRCLE.</u> | <u>RING.</u> | <u>U-SHAPED.</u> | <u>2-CUP.</u> | <u>TOTAL.</u> |
|-----------------|----------------------|--------------|------------------|---------------|---------------|
| | 461 | 109 | 17 | 0 | 587 |

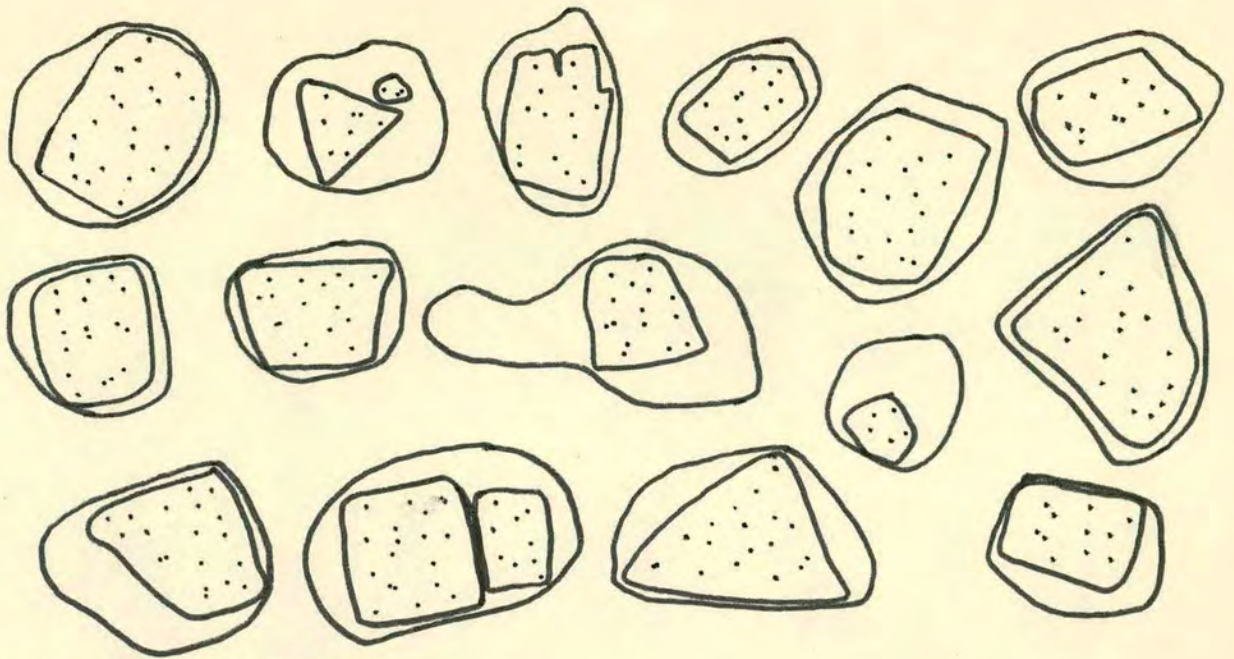
The figures in Table 1 were taken from counts made directly from the image on the screen of the electron microscope. Five sections from five different blocks fixed at the same time were examined. Some of the solid circular profiles were likely to be sections through the cup shaped mitochondria as serial sections showed solid profiles of mitochondria in one section and hollow profiles of the same mitochondria in another section. Plates 13C & 13D illustrate this point. Serial sections also showed a ring shaped mitochondrion in one section (Plate 15A) and a U-shaped profile of the same mitochondrion in another section (Plate 15B). The figures in Table 1 together with the results from serial sections suggest that quite a high percentage of all the

mitochondria in the freshly dug mature tuber were cup shaped. Cup shaped mitochondria have been recorded in a number of animal cells (eg. Stephens and Bils, 1965; Christensen and Chapman, 1959).

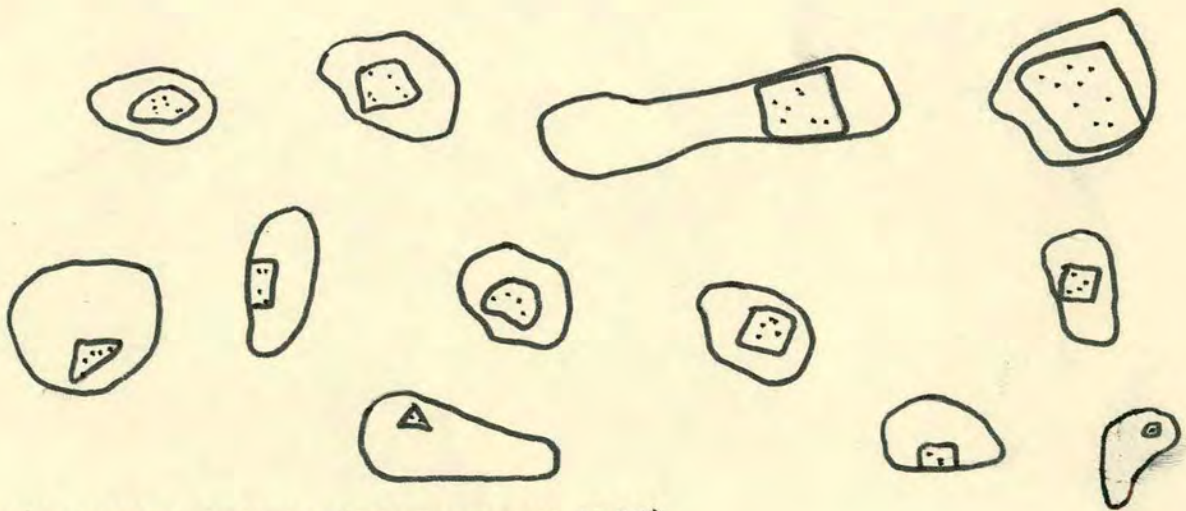
The space within the cup was occupied by cytoplasm which was commonly less electron dense than the cytoplasm in which the mitochondria were lying. (Plates 14A, 14D & 15B) (A similar condition was observed in in the plastids). Both the inner and the outer surfaces of the cup were bounded by the typical envelope of two membranes. The two membranes on the inner and outer surfaces converged towards the base and became incorporated into a narrow sheet. In the centre of the sheet was a thin layer of mitochondrial matrix but no cristae.

d) Crystal- containing bodies.

Thornton and Thimann (1964) first described "crystal-containing bodies" (CCBs) in the subepidermal cells of oat coleoptiles. Since then they have been described further in Avena (Cronshaw, 1964; Cronshaw and Bouck, 1965; O'Brien and Thimann, 1967), and there have been a number of similar organelles described in other plant tissues (Gerola and Bassi, 1964; Bouck, 1965; Kolehmainen, Zech and von Wettstein, 1965; Marinos, 1965; Walles, 1965; Price, 1966; Arnott and Smith, 1967; Jensen and Valdevinos, 1967; Petzold, 1967; Villiers, 1967; Frederick, Newcomb, Vigil and Wergin, 1968). Gerola and Bassi (1964) detected CCBs, which they called proteosomes, in artichoke tuber cells. They did not detect them in freshly excised tissue but after experimentally induced greening of cultured tissue. However, in this investigation CCBs were found scattered in the cytoplasm of tuber cells both before and after culture. They did not appear to have a close association with endoplasmic reticulum.



a) UNCULTURED MATERIAL



b) CULTURED MATERIAL (FIRST DIVISION PEAK)

☐ crystal

FIGURE 2. Tracings of crystal-containing bodies from electronmicrographs.
X 40,000

The CCBs (Plate 15C) in this investigation were approximately circular or oval in profile. They were surrounded by a single membrane which was usually crenate and enclosed a fairly homogenous matrix of intermediate density and an electron dense crystalline body. Usually there was only one crystal per body but occasionally two were seen. In this case the planes of crystallization were usually different in the two crystals. (Plate 15D). Outlines of the profiles of CCBs in cultured and uncultured material are shown in Figure 2. In unstored, uncultured material the CCB was 500 - 700 μ in diameter and the longest dimension of the crystal was 375 - 700 μ . The crystal in many cases occupied nearly all of the interior of the body. The crystal was made up of regularly arranged elementary units with a period of 18 - 20 μ . This is longer than the period of 150 \AA (15 μ) which was given by Gerola and Bassi (1964) for artichoke tuber tissue and the period of 125 - 160 \AA (12.5 - 16 μ) given by Thornton and Thimann (1964) for the oat coleoptile.

A membrane bounding the CCB was always detectable although frequently some portions of it were tangential or oblique to the plane of section causing the membrane to appear indistinct and the boundary diffuse. O'Brien and Thimann (1967) reported that if the membrane of the CCB appeared to be intact then the cytoplasm was well preserved and the ground substance was moderately dense. However if the membrane of the CCB appeared to be missing or ruptured, the ground substance also appeared to be 'badly extracted'. No correlation between the state of the membrane and the state of the cytoplasm was observed in this investigation.

The crystals were also visible when sections of material fixed with glutaraldehyde and osmium were treated with hydrogen peroxide

to remove bound osmium and were examined unstained. Unlike the phytoferritin crystals these crystals were still visible when sections were pretreated with 10% hydrochloric acid.

e) Lipid bodies.

Droplets of material (up to 1 μ in diameter) presumed to be lipid (L) were found in the cytoplasm. (Plate 16A). They occurred singly and in small groups scattered amongst other organelles. However the bodies did not appear to be structurally associated in anyway with these organelles.

The bodies were not delimited by any membrane. They had a uniform electron density and showed no substructure, although they frequently showed signs of "chatter".

It was concluded that these bodies were lipid after sections of tuber material which had been centrifuged for 24 hours at 50,000g (material produced by Dr. M. M. Yeoman.) were examined in the electron microscope. These bodies were all found to have moved to the centrifugal pole of the cells. (Plate 16B).

f) Endoplasmic reticulum and Ribosomes.

The membranes of the endoplasmic reticulum (ER) were dispersed throughout nearly all the cytoplasm. The ER was differentiated into the smooth and the rough forms. In large areas of cytoplasm, such as were found near the nucleus and sometimes in the corners of the cell, the membrane system occurred both as a highly-branched tubular smooth form and as short cisternal elements of rough ER. In the narrow bands of peripheral cytoplasm and even between the nuclei and the plasmalemma

profiles of long cisternae of ER studded with ribosomes lay parallel to the cell wall (Plate 3A). Sometimes only a single cisternae (Plate 3A) was observed but more commonly the sheets were found in stacks (Plate 16C).

A few connections between the ER and the nuclear envelope have been found but no unequivocal connections between the ER and the plasma-lemma, tonoplast or other organelle.

In some cells parts of the tonoplast were partially detached (Plate 15C & 18A (Cell on right)) or were missing (Plate 16A & 18A (cell on left)) and in these cases a row of vesicles assumed to be derived from swollen ER were usually seen at the surface of the cytoplasm. The swelling of the ER is thought to be an artefact associated with the detachment of the tonoplast during preparative procedures.

Ribosomes(R) occurred both free in the cytoplasmic ground substance and bound to the membranes of the ER. A few also occurred on the outer membrane of the nuclear envelope. An occasional helical cluster of ribosomes was seen in the ground substance (arrowed in Plate 7B).

g) Microtubules.

Only a few microtubules(Mt) of the type described by Ledbetter and Porter(1963) were seen in these quiescent parenchymatous cells. They (25 μ in diameter) were found in small groups, one tubule wide, in the cytoplasm adjacent to the plasmalemma (Plates 9B & 16A). No microtubules were found deeper in the cytoplasm or near the nucleus.

h) Dictyosomes.

Dictyosomes (D) (Plates 13B, 17A, B, & 18A.) were found singly and in small groups in the cytoplasm. In profile they were composed

of 1 - 13 fenestrated lamellae or cisternae piled one on top of another (Plate 17A). According to Cunningham, Morré and Mollenhauer (1966) the cisternae are joined by some banding constituent to each other within any one dictyosome. Intercisternal fibres have not been seen in direct contact with cisternal membranes (Mollenhauer and Morré, 1966). Tubular connections between adjacent dictyosomes have been reported (Mollenhauer, 1965; Morré, Mollenhauer and Chambers, 1965.) but these are rare. No such connections were seen in this investigation.

The dictyosomes were always associated with a number of vesicles (Plate 13B, 17A & B.) but polarity across the organelle such as described by Mollenhauer and Whaley (1963) in the maize root tip and by Amelunxen and Gronau (1966) in Acorus calamus was not seen.

Many of the dictyosomes had curved cisternae which often formed 1 - 4 complete rings (Plate 17B). Morré and Mollenhauer (1964) obtained cisternae of this type in isolated dictyosomes and suggested that the ends of the cisternae could fuse.

The appearance of these cisternae in quiescent tuber cells agrees with observations made by Mollenhauer and Morré (1966). They stated that curved cisternae were often found in quiescent cells and also that there were fewer cisternae per dictyosome and fewer vesicles in quiescent cells. Ring like dictyosomes have also been seen as a stage in cyclic changes of the dictyosomes during microsporogenesis in Tradescantia paludosa. (Maryama, 1965). Dictyosomes can also be experimentally modified, some enlarge or curl giving rise to concentric or semicircular cisternal configurations after extended carbon dioxide or potassium cyanide treatment (Mollenhauer and Morré, 1964).

1) Plasmalemma and Associated Structures.

The outer surface of the cytoplasm was bounded by the plasmalemma (Pl). This frequently showed its triple layered nature (Plates 15B,C & D.). The plasmalemma was not applied smoothly and uniformly to the wall but followed a very tortuous path.

Structures associated with the plasmalemma in fungi were first reported by Girbardt (1958),(1961) and similar structures were termed lomasomes by Moore and McAlear (1961). They suggested that lomasomes were formed from cytoplasmic vesicles which fused with the plasmalemma. Peyton and Bowen(1963) however suggested that elaboration of the plasmalemma gave rise to lomasomes. In the tuber tissue there was some suggestion of vesicles either budding off or fusing with the plasmalemma (indicated by arrows in Plates 4,18A.) and some of the vesicles were bounded by a membrane similar in appearance to the plasmalemma (Plate 17C).

Bodies similar to lomasomes are not restricted to the fungi and have been reported in algae (Bouck,1962; Barton,1965; Crawley,1965.) and in higher plants (Monacha and Shaw,1964; Cronshaw and Bouck,1965; Esau,Cheadle and Gill, 1966; Walker and Bisalputra,1967)). A variety of names besides lomasomes have been applied to these structures in the literature; e.g. Charasomes (Barton,1965.); Boundary formations (Esau et al.,1966.).

Marchant and Robards (1968) suggested a new classification for vesicular and membranous structures situated between the plasmalemma and the cell wall. They suggested that these structures should also be called paramural bodies but dividdd these into lomasomes and plasmalemmasomes. "Lomasomes are derived from cytoplasmic membranes, while plasmalemmasomes are formed entirely from the plasmalemma." In this

investigation a variety of material was found between the plasmalemma and the cell wall. The edge of the cell wall had a very fine fibrillar structure (Plate 9B.) and the fibrils were often fairly dispersed. Vesicles and other membranous structures (Plates 9A,B,15D,17C,18A & B.) were also observed and will be referred to here as paramural bodies (PB) (Marchant and Robards, 1968.). Many of the vesicles were delimited by a triple layered membrane similar to the plasmalemma (Plate ~~15D~~ & 17C.). However as the derivation of the vesicles and membranes in the paramural bodies was not determined, classification into lomasomes and plasmalemmasomes is not possible.

The vesicles were sometimes very scattered (Plate 9B) but in other places were grouped within large invaginations of the plasmalemma (Plate 17C). These structures were commonly found associated with compound plasmodesmata (Pd) (Plate 18B). Some of them contained branching, tubular and myelin-like membranous structures and fibrous material as well as vesicles.

j) Tonoplast and Vacuole.

The parenchyma cells had a large central vacuole (Plates 1B & C) but the ring of peripheral cytoplasm also contained numerous smaller vacuoles particularly in the corners of the cell (Plate 2A). A well defined tonoplast (T) limited the vacuoles but in this investigation it was rarely preserved intact. Large and small areas of cytoplasm often appeared in direct contact with the vacuole (Plates 16A & 18A). In such cases an irregular array of membranes could often be seen in the vacuole. These membranes were possibly remnants of tonoplast dissociated from the cytoplasm during preparative procedures. Sometimes a long profile of

detached tonoplast could be identified as at one end it was still continuous with the undetached tonoplast (Plate 18A).

This detachment of the tonoplast has also been reported by O'Brien (1967) in cells of the oat coleoptile who assumes that it is probably an artefact and Buvat (1963) in sieve tubes who reported that it was probably a characteristic feature of this material.

The vacuole (V) usually appeared empty but occasionally contained small amounts of electron dense fibrillar material (Plates 9B and 14E) and a few unidentified bodies (UB) (Plate 17C).



2. Stored tubers.

The ultrastructure of the cells taken from the firm, unsprouted stored tubers was found to be similar to that of the cells taken from freshly dug tubers.

From May onwards the stored tubers softened and sprouted and in these tubers some changes in ultrastructure were observed. Changes were detected in the membranous systems of the plastids. Often the central system was in the form of a regular corpo opaco and in some of the plastids a few regular tuber elements (approximately 20 μ in diameter) were found to be associated with the central system (Plates 19A and B). Similar structures were also found in plastids in the adventitious roots from the sprouting tubers (Result section IB 4). These structures were slightly smaller than prolamellar body units (30 μ in diameter) which were found in etiolated leaves and stems from sprouting tubers (Sections IB 2 and IB 3 respectively).

The peripheral system formed a very regular network in many plastids (Plate 19C).

3. Immature tubers.

The immature tubers were taken from plants growing outside whose aerial parts were green. ~~outside.~~ Several differences were observed in the ultrastructure of the plastids and mitochondria in cells

from immature tubers compared with those in cells from mature tubers. The cells from the immature tubers were smaller than the mature ones and less vacuolated. Unfortunately no stages in the development of clusters of plastids were observed in these cells. The clusters observed were similar to those found in the mature highly vacuolated tissue. The membranous systems of the plastids were also similar to those in the mature tubers. However, as already recorded in Section IA 1b on plastids in freshly dug mature tubers, the plastids contained a large proportion of phytoferritin deposited in crystals (Plate 20A). The plastids also contained granules similar to starch (Plate 20A). The aerial parts of the plants from which the immature tubers were taken were green and presumably photosynthetic. Most of the carbohydrate stored in the tubers is in the form of inulin but apparently starch is also stored. Starch is possibly only formed when the amount of sugar supplied from the shoot exceeds that utilized in the synthesis of inulin. (The plastids in cultured tissue (eg. Plates 31B and 33A) and green leaves (Plate 21) of this material were also found to contain granules similar to starch). The position of inulin in the cells is not known although it is commonly assumed to be in the vacuole.

The mitochondria in the immature tubers were small and circular or oval in outline (Plate 20B). Many of them showed invaginations in the surface which in some cases appeared to extend into cavities in the centre of the mitochondria (Plate 20B). The mitochondria were enveloped by two membranes which continued into the invaginations and into the cavities when these were present. However no basal sheet of envelope membranes without cristae was found as in the mitochondria in the mature tuber cells.

B. Other Organs.

To aid in the interpretation of the ultrastructure of the plastids of the tuber, particularly with regard to the membrane systems, the plastids in other tissues were examined. The other tissues included green leaves, etiolated leaves and shoots, and adventitious roots from Jerusalem artichoke plants.

1. Green fully expanded leaf.

Three cell types were examined in green, fully expanded leaves. The cell types were mesophyll, vascular parenchyma and epidermal. The plastids of the mesophyll cells (Plate 21) were typical chloroplasts. Thylakoids of relatively uniform width (18 μ) were present both as stroma lamellae and organised into grana. The plastids in each cell type had two membrane-bound systems. Peripherally there were a few profiles of cisternae of more variable width (15-50 μ). The membranes of some of these were continuous with the inner envelope membrane. A distinct tubular system, as found in the tubers, was not recognised.

In the cells of the vascular sheath (Plate 22A) the thylakoidal system was similar to that of the mesophyll chloroplast but was less extensive. There was also a well developed peripheral system consisting of cisternae with contents less dense than the stroma. The cisternae were of irregular width (15-100 μ), often flattened and lying parallel to the envelope. In these plastids, tubules, like those of the third system in tuber plastids were abundant.

In the plastids of the epidermis (Plate 22B) the thylakoidal system consisted of strings of grana each composed of two discs joined by short lengths of stroma lamellae. Peripherally, parallel to the envelope, there were flattened cisternae, up to three deep, like those of the vascular sheath plastids.

Large starch granules were found in plastids in mesophyll cells and in guard cells but never in plastids in vascular parenchyma cells or in other epidermal cells. Phytoferritin was occasionally found in plastids in mesophyll cells.

2. Etiolated leaf.

Most of the plastids in cells from etiolated leaves (Plates ~~23A and B~~) contained a regular corpo opaco and a crystalline prolamellar body, which in many micrographs appeared to be joined to the corpo opaco or other part of the central system (Plates ~~23A and B~~). The crystalline prolamellar body and the parts of the central system similar to those recognised in tuber plastids had contents of similar electron density. They are both assumed to be part of the central membranous system. Associated with the central system were 'ribs', 10-20 μ m in diameter, lying often parallel to one another in the stroma adjacent to the surface of parts of the central system. (Plates ~~23A and B~~). The ribs were characteristically observed as a lining to shallow or deep pockets of stroma material in the central system. The ribs were also observed in plastids in etiolated stems and in cultured tuber tissue (Section IIB 1a). Distinctive peripheral and tubular systems were also found in the plastids together with phytoferritin, in all its previously described morphological forms (Section IA 1b), and starch granules.

3. Etiolated stems.

The plastids in etiolated stems (Plate 24A) growing from tubers had a recognisable central system, consisting of a corpo opaco and crystalline prolamellar body. "Ribs" were often associated with the central system as in the etiolated leaves. The plastids also

contained a peripheral system, a tubular system, phytoferritin and starch granules.

Secretory ducts were frequently detected in sections of etiolated stems (Plate 24B) and the plastids in the secretory cells surrounding the ducts were found to be distinct from the other plastids in the stem. The plastids were closely sheathed with endoplasmic reticulum (Plates 25A,B and C). Wooding and Northcote (1965) made observations on a similar phenomenon in developing sieve tubes of Acer pseudoplatanus and resin canal cells from Pinus picea. They suggested that in the resin canal cells the sheath may play an important function in the synthesis of the predominantly terpenoid resin. The secretory cells of the artichoke were found to contain lipid material. At the light microscope level sections of material treated with Sudan III were seen to contain a red stain in the ducts and surrounding cells. However whether the endoplasmic reticulum and the plastids were associated with the synthesis and transport of the lipid is not known. The plastids in these artichoke cells did not contain a regular corpo opaco but an irregularly branched central system (and associated ribs). A peripheral system and tubular system were both observed. The plastids also contained a structure which was not detected in any of the other plastids observed during this investigation. This structure was made up of straight filaments (approximately 10 μ in diameter) which were orthogonally arranged in the stroma. Plate 25B shows them in cross section and Plate 25C shows a longitudinal section of the filaments. The significance of these filaments is at present obscure.

4. Adventitious roots.

In the root, plastids similar to those of the tuber were present but several of the plastids (Plates 26A,B and C) contained structures which appeared to be an elaboration of the third tubular system. Similar aggregates of tubular structures have been reported in floral glands of Passiflora by Schnepf (1961), in plastids of the cotton nucellus by Jensen (1965) and in proplastids of Bryophyllum and Kalanchoe shoot apices by Gifford and Steward (1968). The latter suggested that spherical membrane-bound inclusions in the plastids arise by the accumulation of material within the cisternae of the tubular lamellae. The inclusions are thought to be then transferred to the vacuoles. No such accumulation and transfer of material was detected in the artichoke. Some of the plastids contained a few tubular elements (Plate 26D), similar to those found in stored tubers (Section IA 2 which were possibly connected to the central system. The central system was frequently a regular corpo opaco (Plate 26 D).

SECTION II. CULTURED TUBER CELLS.

Observations were made on tissue which was cultured for up to 6 weeks. During the initial period of culture, a fairly uniform population of cells underwent a synchronous division and the changes occurring during the dedifferentiation of the tuber cells were recorded as a developmental sequence. However as the culture period increased the population of cells became more variable and the cell divisions were asynchronous. Therefore changes recorded in cells cultured for long periods did not necessarily represent changes in the explant as a whole. The observations reported here are mainly concerned with plastid and mitochondrial structure. The results in this section can therefore be divided into two parts : A. Changes occurring during short term culture and B. changes occurring during long term culture.

A. Changes occurring during short term culture.

This section contains the results of a preliminary ultra-structural survey of the culture period up to the end of the first division. It is this initial period of culture which has been studied most in this department, (Yeoman, Evans and Naik, 1966; Yeoman and Evans, 1967; Mitchell, 1967; Fraser, Loening and Yeoman, 1967), and the electron microscopic investigation was performed in order to characterize the system in more detail.

Observations were made on slices of tissue removed from the periphery of the explant as it was in the cells of these peripheral layers that the first partially synchronous cell division occurred. It was assumed that changes in the cells associated with this division must

also be fairly synchronous. However it is not known whether all the ultrastructural changes detected were concerned with the division. Not every cell divided but dividing and non-dividing cells could not be distinguished by their ultrastructure before the commencement of phragmosome formation. The changes observed before phragmosome formation appeared to occur in all cells whereas the phragmosome appeared to develop only in cells beginning to divide. The division occurred after about 24 hours culture at 25°C but the time varied according to the length of storage of the tubers. However it is important to note that despite the variations in the predivision time the length of the combined S, G₂ and mitotic phases is a constant (Mitchell, 1967).

The results present a rather incomplete record owing to the large size of the cells (the entire lengths of which were not sectioned) and to difficulties experienced in the preparation of material for electron microscopy; sections of some batches of material were found to only consist of cell walls, a few scattered organelles and an assortment of membranes. No sections could be cut of other batches of material as they did not embed satisfactorily. Therefore many of the time intervals between successful preparations in any one experiment were longer than desired.

The results are presented in a developmental sequence ; however it is difficult to connect all the information obtained on a single time scale because of the gaps in the sampling sequence, and the variation in the time of the first division. Therefore actual times when given must be considered as approximate.

Short term culture has been divided into five sections:

1. The Rind.
2. Changes occurring before phragmosome formation.
3. The phragmosome (Early prophase).
4. Mitosis (Late prophase-telophase).
5. Cytokinesis

1. The Rind.

The periphery of every explant was surrounded by cells cut and damaged during its excision from the whole tuber. After a few hours culture changes were recognised in these cells. They contained bodies similar in appearance to lipid bodies, various unidentifiable membranous bodies and often electron dense material (Plate 27A). As culture progressed these cells appeared to lose their contents and the cell walls in this outer layer collapsed against each other. This layer is referred to as the rind.

The cells immediately below the obviously damaged cells usually contained a ground substance of greater electron density than the cells in the deeper layers. Division stages were found in a few of these cells.

Yeoman, Naik and Robertson (1968) suggested that the presence of the damaged peripheral cells regulated the proportion of cells which divided. They suggested that cells damaged during excision undergo autolysis and that the products of this autolysis pass out into the culture medium and also into deeper layers of cells. Certain of these autolytic products, in conjunction with 2,4D are then thought to stimulate cell division. Sheldrake and Northcote (1968) investigated the production of auxin by autolysing tissues. They suggested that the production of auxin in dying cells may be important in stimulating cell division in other

cells in the tissue. This may be the case in the artichoke tuber explants but the autolytic products from the damaged peripheral cells have not been isolated and identified.

Both acid and alkaline phosphatase activity have been demonstrated by histochemical techniques in the cells of the outer layers of the explant after several hours culture (Robertson, 1966). These enzymes are closely connected with autolysis (e.g. Gahan and Maple, 1966). Berjak (1968) reported that acid phosphatase was found in membrane bound bodies and he suggested that the release of hydrolytic enzymes accompanies the senescence of old root cap cells in Zea mays.

During this investigation acid phosphatase activity was detected at the ultrastructural level and the rind exhibited a positive diffuse reaction. In the outermost cells in which the various organelles could be identified the activity was found to be associated with the cisternae and vesicles of dictyosomes (Plate 28A) and with other vesicles possibly derived from dictyosomes. Activity also appeared to be associated with the endoplasmic reticulum (Plate 28B), the plasmalemma, the nuclear membrane and the nucleus (Plate 28C). No activity appeared to be associated with plastids, mitochondria or crystal-containing bodies. The activity located in the rind and nuclei was composed of large electron dense deposits whereas the activity associated with the dictyosomes and other organelles was finely granular. Mitchell (personal communication) found that the acid phosphatase activity was particulate during early culture but later became more diffuse. The large deposits may represent diffuse activity and the fine granules particulate activity.

Previous reports suggesting an association of acid phosphatase

activity with dictyosomes include Pour (1963), Novikoff, Hirsh and Quintana (1965) and Pickett-Heaps (1967). Pickett-Heaps reported that the degree of reactions was progressively greater across the stack of cisternae but this was not found in the tuber cells. Pickett-Heaps also found phosphatase activity of varying degrees in the wall, nucleus and often the vacuoles of the wheat root cells. Catesson and Ganinski (1967) observed acid phosphatase activity in the nuclear membrane and endoplasmic reticulum.

The characteristic ultrastructural appearance of the outer cells is likely to be the result of autolysis.

The rind was found to persist throughout the whole culture period (Plate 58). It increased in thickness over the first 1-2 weeks and therefore it is assumed that outer cells were continually dying and being added to the rind. After 3-4 weeks culture the rind often appeared to have been disrupted by the growth of cells beneath it. Pieces of rind may then have flaked off.

2) Changes occurring before phragmosome formation.

The first change detected in the ultrastructure of the parenchymatous cells was the appearance of clusters of ribosomes in the form of spirals and helices (Plate 29A and B). The spirals were always found associated with endoplasmic reticulum and profiles of rough endoplasmic reticulum were more abundant in cultured material. The helices were found scattered in the ground substance. Clusters of ribosomes have been called polysomes in the literature (e.g. Bonnett and Newcomb, 1965) and will be referred to as such in this thesis. Polysomes are defined as aggregates of ribosomes joined by messenger RNA. However it has not been demonstrated that the spirals and helices

of ribosomes seen in the electron microscope are bound to each other by messenger RNA (Loening, 1968). The earliest sample taken was after 3 hours culture and a few polysomes were observed in these cells. The polysomes appeared to increase in abundance during the next few hours culture.

Another early change, which was detected after 6 hours culture, was the appearance of large quantities of unidentified electron dense material in many of the cell vacuoles. The material was often dispersed and fibrillar in nature as can be seen in the vacuoles of Plates 30 and 31A. However profiles of rounded electron dense bodies were also seen. Some of these (I) resembled lipid bodies both in their homogenous appearance and in the fact that they often were shattered (Plates 30 and 31A). Other bodies (II) were less solid and had fibrillar contents (Plates 29A, 32A, B and C). These bodies were found in the cytoplasm (Plate 32A) as well as in the vacuole.

Many cells were found to have a very electron dense ground substance (Plates 31A, 32B (bottom cell) 33A) which partially obscured the appearance of the membranous organelles. Myelin-like bodies (MB) were frequently found in these cells especially near plastids and nuclei (Plates 31A, B and 33A). A few also appeared to lie between the plasma-lemma and the cell wall (Plate 33B).

Clusters of plastids were still frequently found in cultured material before the first cell division. After 6 hours culture, small clear circular areas (S) were seen in the stroma of the plastids (Plates 31B and 33A). These clear areas resemble the bodies referred to as starch in the literature (e.g. Marinos, 1967); (Newcomb, 1967). The bodies in the cultured tuber cells are also assumed to contain starch as a substance which gave a positive reaction with periodic acid-schiff was

detected at the light microscope level, in cultured cells. The number and size of these starch bodies appeared to increase over about the first 3 days of culture.

After about 12 hours culture phytoferritin was not generally detected in the plastid stroma. The variation in the electron density of the stroma, even between adjacent plastids, was found to be very great during the first few days culture (Plate 29B).

During short term culture only flat (and therefore straight in transverse section) cisternae of dictyosomes were seen (Plates 34A and B). The dictyosomes were often grouped (Plate 34B) and appeared to be more numerous than in the uncultured material. The dictyosomes were always associated with many vesicles of varying sizes (Plate 29A).

Most of the mitochondrial profiles were fairly simple and small (0.5 - 1 μ m in diameter) (Plates 31A,B,32B and 36B). Very few cup shaped mitochondria were observed. The number of cristae per mitochondrial profile did not change significantly during short term culture. Many mitochondria contained an electron transparent ground substance and few cristae (Plate 35A) in contrast to the denser mitochondria in uncultured tissue. During this period of culture the mitochondrial profile area, in one experiment at least (see Appendix I), increased. Therefore the less dense appearance of cultured mitochondria may be the result of an increase in the total volume without an accompanying increase in cristae number and stroma material.

The circular electron dense bodies, which appeared to consist of a whorl of membranes were commonly found in mitochondria in material which had been cultured for a short period.

The crystal-containing bodies (CCBs) were found to be smaller in material cultured for 12 hours or more (Plate 34A). Their size in cultured material can be compared with their size in uncultured material in Figure 2 which shows tracings of CCBs taken from electron micrographs (x 40,000).

An increasing number of microtubules were detected near the plasmalemma in cultured cells before the first division (Plates 35A and B) but large numbers such as those found by Pickett-Heaps and Northcote (1966b,C) in their "preprophase band" were not detected. However as serial sections were not cut through the entire length of these large cells a preprophase band could still have existed but have remained undetected.

3) The phragmosome (Early prophase).

In uncultured material the nucleus was found to be flattened between the vacuole and the cell wall in a very narrow band of peripheral cytoplasm but the dividing nuclei in these large cells were always found away from the cell wall and towards the centre of the cell. A variety of nuclear positions and cytoplasmic arrangements between these two extremes were found and have been arbitrarily arranged in a series which is thought to represent the sequence of events (Figure 3). However it is important to point out that the arrangement of some of these profiles may not form a developmental sequence but represent sections cut in different planes through cells which had similar arrangements of cytoplasm.

The first changes noticed in the nucleus were that it assumed a more rounded profile (Plates 36 and 37) (although it was sometimes very lobed), and did not appear to lie as close to the cell wall as in

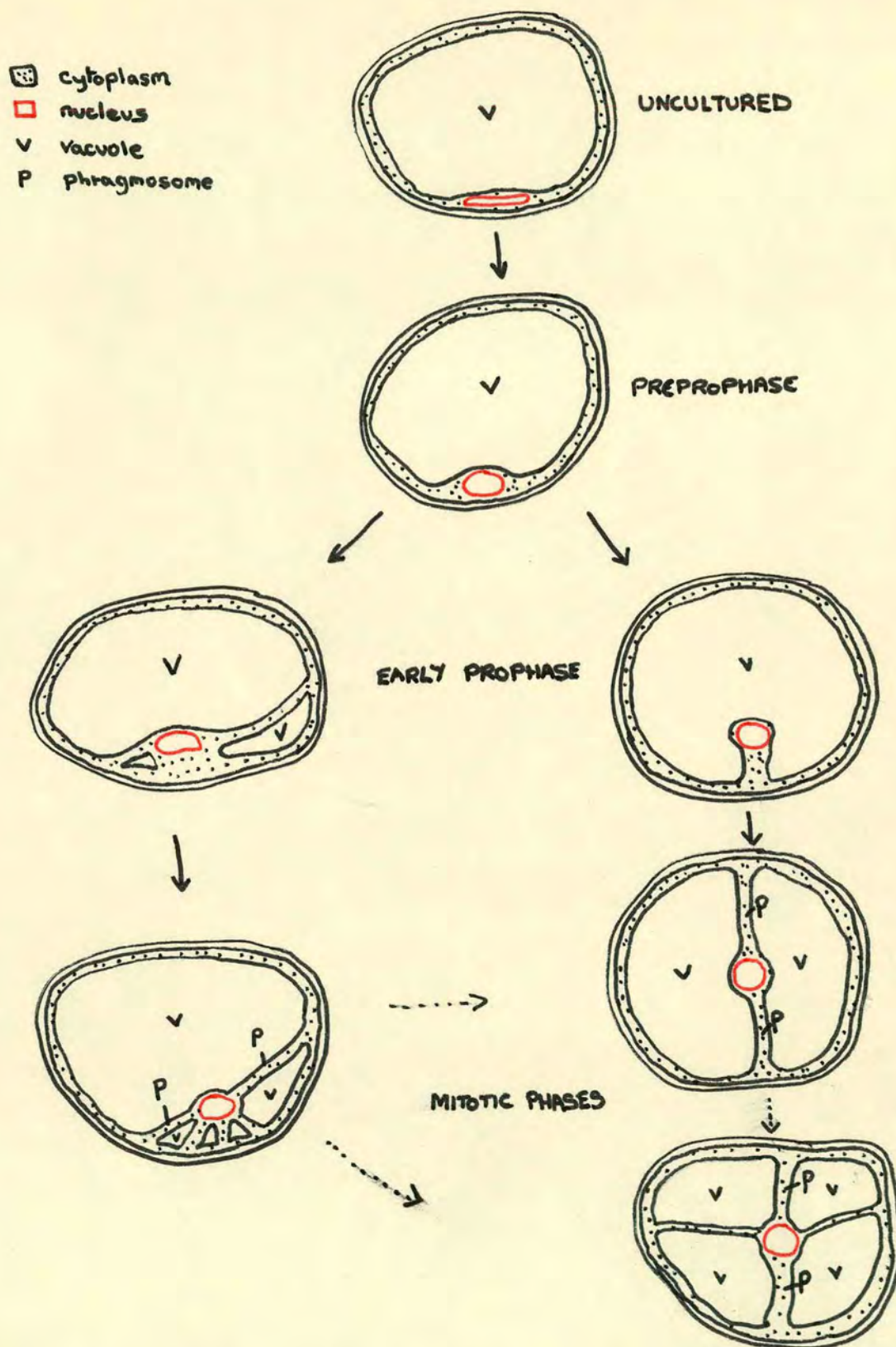


FIGURE 3. Schematic diagram of changes in the arrangement of the cytoplasm and the position of the nucleus during the first cell division.

the uncultured cells. Dense aggregates of chromatin were more frequently observed in these nuclei (Plate 36) than in uncultured ones. The change in the shape of the nucleus was accompanied by nucleolar changes. The nucleolus appeared to be less compact and the fibrillar and the granular regions were intermingled (Plates 36 and 37). There was often a very large more electron transparent central region observed in the nucleolus (Plate 37). Granular material surrounded this region and granular particles, similar in size to ribosomes, were found in it. Fibrillar material like the chromatin outside the nucleolus was also found in it. Smaller electron transparent regions within fibrillar zones surrounded the central one (Plate 37). They contained only fibrils. A few karyosome-like bodies could still sometimes be found at this stage (Plate 36).

Cells in which the nuclei had changed their shape and position, and in which nucleolar changes were detected are assumed to have been in early prophase.

Nuclei, whatever their position in the cell, were always surrounded by cytoplasm, the perinuclear cytoplasm. In many of the early prophase nuclei positioned near the cell wall the perinuclear cytoplasm was connected to cytoplasmic strands on the side away from the wall (see arrow in Plate 37). These strands may have been connected to peripheral cytoplasm in other parts of the cell and may have been concerned with the movement of the nucleus away from the wall. Profiles of nuclei at varying distances from the cell wall and various patterns of cytoplasm were obtained. Some of these are shown diagrammatically in Figure 3. Many profiles showed connections of cytoplasm between the

the cell wall and the nucleus in one area only (Plate 38). It is probable that this area was where the nucleus originally lay. Some of these profiles showed a stout column of cytoplasm between the nucleus and the cell wall (Plate 38), whilst others showed several, often narrower strands separated by sections of the vacuole between the nucleus and the cell wall (Plate 40). In contrast to these profiles many nuclei were found in the centre of a cytoplasmic strand traversing the whole cell (Plate 41). The direction of this strand was nearly always similar to the direction of the majority of cell plates and new cell walls in cells which had completed the first division. The majority of the cells divided anticlinally at this division. Similar cytoplasmic strands in vacuolated cells were termed the phragmosome by Sinnott and Bloch (1940). They found that the expanding cell plate followed exactly the course of the phragmosome so that this structure occupied the position where the new cell wall would later be laid down.

Many vesicles (of various sizes and density), dictyosomes, endoplasmic reticulum, polysomes, mitochondria, plastids and a few CCB'S were found in the cytoplasmic strands and in the perinuclear cytoplasm. In the strands all the organelles were orientated so that the largest dimension appeared to be parallel with the direction of the strand (Plate 39 and 42).

Another structure, which does not appear to have been described before, was found connected to the nucleus in the perinuclear cytoplasm, and in the cytoplasmic strands up to a distance of 10 μ from the nucleus. Serial sections showed that many of these structures in the perinuclear cytoplasm and cytoplasmic strands were also connected to the

TABLE 2

| CELL TYPE | Total No. nuclei observed | No. of cells containing nuclear extensions | No. of cells containin microtubules near extensions |
|---|------------------------------|---|---|
| Nucleus next to wall; Cytoplasmic strands absent. | 49 | 0 | 0 |
| Nucleus next to wall; Cytoplasmic strands present. | 3 | 3 | 0 |
| Nucleus away from wall; Cytoplasmic strands present. | 58 | 28 | 19 |
| Mitotic phases after breakdown of nuclear envelope. | 25 | 0 | 0 |

Correlation of the position of the nucleus and the presence of cytoplasmic strands with nuclear extensions and microtubules in cells from cultured tissue.

the nucleus but whether all similar structures were so connected is not known. This structure is termed a nuclear extension (NX) in this thesis. It is a long tube, the wall of which is composed of two membranes continuous with the nuclear envelope and surrounding homogenous material also continuous with the nucleus. Aggregations of chromatin were never seen in the nuclear extensions. Connections between this tubular organelle and the nucleus can be seen at the arrows in Plates 39 and 43. The diameter of the whole tube (including membranes) was 33-75 μ and the width between the inner and outer membranes was 13-23 μ (cf. distance between two membranes of nuclear envelope 18-30 μ). The outer membrane of the nuclear extensions, like that of the nucleus, was connected to endoplasmic reticulum (red arrow in Plate 39, 42 and 44) but ribosomes were not detected on it. The structure was occasionally found to branch (Plate 45A) and was frequently associated with microtubules (Plates 43, 44, 45A,B and Table 2). Table 2 shows the relationship between the occurrence of nuclear extensions and cytoplasmic strands in cultured cells. No nuclear extensions were detected in cells without cytoplasmic strands but in 58% of the sections observed of early prophase cells containing cytoplasmic strands nuclear extensions were found. Several extensions or bits of extensions were sometimes found in one cell and they always lay in the direction of the cytoplasmic strand (Plates 39, 42 and 45B). However cells in later stages of divisions were not found to contain these nuclear extensions. It is important to note that these nuclear extensions were not only found in early prophase material at the first division (Plates 39, 42, 44 and 45B) but also at the second division and in tissue cultured for varying lengths of time which contained nuclei,

assumed to be in an early prophase state, suspended in the centre of highly vacuolated cells by cytoplasmic strands. Plates 43 and 45A were taken of a cell in tissue cultured for 7 days. Nuclear extensions were found in tissue cultured on all three media (Methods;Section IB) and under a variety of light conditions.

Prophase nuclei were frequently surrounded by microtubules and small vesicles (Plate 44). The microtubules presumably form the nuclear spindle when the nuclear membrane breaks down. Whether the microtubules which were found in the cytoplasmic strands near nuclear extensions are also involved in spindle formation is not known.

4) Mitosis (Late prophase-telophase).

The sequence of events during mitosis appeared to follow the usual pattern. Only a few cells have been seen in either late prophase, metaphase, anaphase or in early telophase and therefore detailed results of chromosome structure and spindle structure cannot be given.

Mitochondria, dictyosomes, CCBs and plastids appeared to be excluded from the spindle but endoplasmic reticulum and vesicles (which may or may not have been formed on dictyosomes) were seen within the spindle and between the chromosomes.

In several oblique sections of the spindle chromosomes were scattered in the spindle region but without the examination of serial sections it was impossible to know whether they were in metaphase or anaphase (Plate 46). Plate 47A shows a longitudinal section through the spindle at anaphase and it can be seen that the chromatids have separated and moved to opposite, broad poles. The equatorial plate region is magnified in Plate 47B and can be seen to be composed of

microtubules, endoplasmic reticulum, vesicles and ribosomes. The vesicles may have been an early stage in cell plate formation.

Plate 48 shows an early telophase nucleus near the cell plate. The chromosomes have lost their identity and the nuclear membrane has formed around the nucleus. Early telophase nuclei were irregular in shape and in profile contained spaces which were surrounded by two membranes (Plate 49). These spaces may have been continuous with the cytoplasm but serial sections were not cut to verify this suggestion. Myelin-like bodies were also associated with these nuclei. The electron micrographs obtained do not show whether they were inside these membrane bound spaces or were mixed with nuclear material (Plate 49).

The nuclear material in early telophase nuclei was very dispersed. There were large aggregates of fibrous material, but the granular material was dispersed between clumps of chromatin throughout the whole nucleus (Plate 48 and the enlargement Plate 49). The granular material later presumably becomes compacted around the fibrous material. This section is seen in Plate 52 in the nucleus next to a new cell wall. This nucleus also contains a few vesicles but no large spaces and myelin-like bodies.

5) Cytokinesis.

The cell plate and cell wall formation proceeded from the centre of the spindle along the phragmosome towards the parent cell wall. In most cells the first division was anticlinal and sections of cell plate and cell wall were surrounded by very narrow layers of cytoplasm containing an apparently random assortment of organelles.

The cell plate was composed of variously sized vesicles,

(Plate 50), multivesicular bodies (Plate 51A), myelin-like bodies (Plate 51A and B), endoplasmic reticulum and microtubules (Plate 50). Many dictyosomes were found around the cell plate, particularly at the growing end (Plate 51A) and it is assumed that many, if not all, the vesicles were derived from them. The origin of the multivesicular bodies and myelin-like bodies is unknown. The microtubules, possibly the remains of spindle fibres, were only found at the growing edge of the cell plate (Plate 50) but were not always detected (Plate 51A). The smallest vesicles were also seen at the growing edge and it assumed that these small vesicles fused to give the larger vesicles. Many of the larger vesicles contained electron dense fibrillar material (Plates 50 and 51B) and similar fibrillar material was seen in the new cell walls (Plates 52, 53 and 54A). The fused membrane around the vesicles appeared to give rise to the plasmalemma and cell wall (Plates 52, 53 and 54A). Their origin is unknown. Perhaps they were derived from the multivesicular bodies found in cell plates. Some of the paramural bodies also contained fibrillar material and a variety of membranes (Plate 53). Bodies similar to paramural bodies were also found in the cytoplasm (Plate 54B). However several serial sections showed that some of these were really situated between the plasmalemma and cell wall. Perhaps if they had all been serially sectioned they would have been found in this position.

The new cell wall was often not straight in transverse section. This may have been the result of poor fixation. Sutton-Jones and Street (1968) reported that in cultured Acer cells: 'Newly formed cross walls following a zigzag course were frequently observed'. The new thin

wall can be seen meeting the wider parent cell wall in Plates 54A and B. Microtubules were found near the plasmalemma by the newly completed cell walls (Plate 54B).

No details of plasmodesma formation were observed.

B. Changes occurring during long term culture.

The first two synchronous divisions (Yeoman, Evans and Naik, 1966) were followed by rapid asynchronous divisions in the peripheral layers giving rise to regular rows of cells at right angles to the surface of the explant (Plate 55). In liquid culture the divisions were distributed uniformly over the whole surface whereas in agar culture they tended to be restricted to the surfaces not in contact with the medium. During this phase of rapid division the cell size was considerably reduced and the ratio of volume of cytoplasm to vacuole increased. (Cf. Plate 1A with Plate 56). Plates 57A and B are enlargement of parts of the same tissue that is shown in Plate 56. They contrast greatly with the uncultured tissue shown in Plate 1B.

Cut cells on the surface of the explant together with some of the apparently uncut cells below them degenerated (Plate 58) and formed a layer which stained deeply with safranin (Yeoman, Dyer and Robertson, 1965) and was found to be acid phosphatase positive (Section IIA 5).

After about 7 days culture, areas of random division were seen. These gave rise to meristematic nodules (Yeoman, Dyer and Robertson, 1965). Their development was not studied in this investigation. With liquid culture the nodules were scattered uniformly over the whole surface, but with agar media they tended to be restricted to the superficial layers immediately above the surface of the medium. The production of nodules was accompanied by the differentiation of tracheidal elements below them. These can be seen in Plate 57A. Details of their differentiation were not recorded. During the differentiation phase the cell division rate decreased and cells increased in volume and became more fully vacuolated. The surface of the explants became very irregular and the outer cells were often rounded and large intercellular spaces were seen. Robertson (1966) reported that in liquid culture some cells broke away into the medium.

In direct contrast to the short term culture the population of cells within the explant was very variable and changes recorded in cells cultured for long periods do not represent changes recorded in cells in the explant as a whole. The observations reported here were nearly all confined to the outermost files of cells i.e. the newly produced callus cells. A variety of division stages were seen but the results are mainly concerned with plastid and mitochondrial structure.

1. Plastids.

Depending upon the medium and the illumination used in any experiment, cultures either remained non-green or turned green. The ultrastructure of the plastids found under the different conditions will therefore be described under two headings a) Cultures which did not turn

green, and b) cultures which did turn green.

The changes reported in the previous section on short term culture i.e. the appearance of starch and the disappearance of phyto-ferritin appeared to be common to all cultures. In older cultures, 3-4 weeks, small amounts of phytoferritin were again detected. Also clusters of plastids became less commonly observed after the first cell division. There appeared to be fewer clusters per unit volume and since only ^{small} increases in volume occurred during the first two days (Yeoman, personal communication) it is probable that the plastids in some of the existing clusters had dispersed. There was certainly not sufficient formation of new clusters to keep pace with the division of the cells. Plate 59A shows a cluster in a cultured explant. The areas of encircled cytoplasm tended to become fewer and thus simpler plastid shapes were relatively more common during culture. This last feature is in direct contrast to the behaviour of the mitochondria of which increasingly bizarre forms described in Section IIB 2 were found in the older cultures.

a) Cultures which did not turn green.

Cultures grown on standard medium did not turn green even when illuminated. A few cultures grown on all the different media were kept in complete darkness and did not turn green.

The central, peripheral and tubular systems were readily distinguishable in the plastids. The peripheral and tubular systems were similar to those in the plastids of the mature tuber. The central system was again of very variable morphology and two new features were obvious. The first was the appearance in some plastids of lamellae as

part of the central system (Plate 59B) a condition not common in plastids from the uncultured tissue. There was no development of grana even in illuminated cultures. The second was the appearance of 'ribs' similar to those found in etiolated leaves (Section IB 2) and stems (Section IB 3). The ribs as in the etiolated leaves and stems were characteristic as a lining to shallow or deep pockets of stroma material (Plate 60) in the central system, including the stroma lining the inner surface of lamellae from the central system (Plate 61). These loops which were sometimes nested, must represent hollow domes or cylinders in three dimensions. Ribs of this type do not appear to have been described before, although similar loops of lamellae have been illustrated connecting with the prolamellar body in etioplasts (e.g. Wettstein, 1959; Englebrecht and Weier, 1967). Similar loops, again without ribs, have been described by Newcomb (1967) attached to the tubular complex in bean root plastids. The ribs in the artichoke appeared in cross section sometimes with an electron transparent core and sometimes smaller and solid (Plate 61C). The bounding membrane of the central system is sometimes absent in the region adjacent to the ribs. Perhaps the preservation is inadequate with present techniques.

No regular prolamellar body was detected in these cultures even when the explants were cut in dim green light and cultured in complete darkness for several weeks. Plastids from these cultures frequently had a regular corpo opaco.

b) Cultures which did not turn green.

Illuminated explants turned green only when cultured on a medium without coconut milk.

The process of greening in discs of artichoke tuber tissue has been

studied by Gerola and Dassù (1960). They reported that the corpo opaco became divided up by the invagination of its limiting membrane. The fragments so formed developed into both the discs within the grana and also the stroma lamellae. Plate 62A shows the central system in the course of fragmentation and Plate 62B shows a chloroplast with a thylakoidal system presumably derived in this fashion. The grana and stroma lamellae are therefore homologous to the central system.

Again an apparently independent peripheral system was recognisable in these plastids. The plastids also contained a large number of fairly electron dense droplets (up to 100 μ in diameter) and a few starch granules (Plate 62B).

Greening and the development of chloroplasts occurred within three days in some cultures but in others the process was more gradual and took more than a week. This phenomenon may depend upon the time of year at which the experiments are performed. Chloroplast development in three days occurred in early November whereas in January after two months storage chloroplast development did not occur for at least a week and then in only some of the cultures. Storage of the tubers appeared to reduce the greening capacity.

2. Mitochondria.

Profiles of spheres and short rods similar to those seen in sections from uncultured tissue were also apparent in sections from cultured tissue (Plate 63), but in the cultured tissue other forms probably derived from the similar bodies were also observed after a week's culture. Rod like profiles which were considerably longer (up to 13 μ) than the corresponding profiles in the uncultured tissue

were frequent, (Plate 64A). Some of these may represent longitudinal sections through cylindrical rods while some are likely to represent sections through flat plates as long lengths were cut so frequently. The cylindrical rod pattern was frequently elaborated through convolution and branching (Plates 64B, 64C.) In this whole group the width of the basic structures was of the order 1-2 μ . The organelles were enveloped by two membranes and the majority of them contained short cristae embedded in an electron dense matrix. In some of the mitochondria, particularly in tissue that had been cultured for 15 days or more, the cristae were abnormally extended and described a variety of patterns. In some the extended cristae lay along the length of the rod (Plate 65A), and in others they assumed the form of circles (Plates 65B, 65C.) Occasionally fine fibrils were observed parallel to the cristae (Plate 65A). Another structural component possibly related to the extended cristae was seen within some elongate mitochondrial profiles. (Plate 66A). Typically within the mitochondrial envelope there was a peripheral zone occupied by short cristae, but the centre was occupied by a system of electron dense lines separated by clear zones about 4 μ in width (Plate 66B). A transverse section through what may have been a rod suggests that the lines were in fact sections through lamellae (12-13 μ wide) that were folded over each other (Plate 66C). Giacomelli, Wiener and Spiro (1965) reported similar mitochondrial structures in the rat adrenal gland but these structures were found to be composed of tubules and they were thought to connect with the cytoplasm through gaps in the limiting membranes.

In sections from tissue cultured for at least a week a second

group of forms can be distinguished from the first in which spheres rods and plates predominated. The simplest of the profiles from this second group consisted of two oval mitochondrial outlines connected through a fine thread (Plates 62A and B). The profile probably represents a section through a plate, the rim of which had a normal mitochondrial system, but the centre of which consisted of two double membranes separated by a strip of mitochondrial matrix without cristae. This system appears to be related to the cup-like structures found in uncultured tissue. It is also apparently related to another structure (Plate 67C) found in cultured tissue. The profile shows two club-like mitochondrial areas connected through a long narrow structure bent into the form of a loop. This profile probably represents a longitudinal section through a ball shaped structure. The rim of the bell carried normal cristae in a matrix. It was surrounded by an envelope of two membranes which continued into the dome. In this, the envelope was present on the inner and outer surfaces, with a tiny strip of mitochondrial tissue between them. The width of the bell (Plate 67C) is about 3.5μ and the depth about 2 u. In sections a variety of bizarre profiles are seen which are presumably derived from this structure. For example Plates 68A and 68B show two sections of the same mitochondria illustrating two different outlines. The bangle of Plate 68C is probably a section along the level a_1-a_2 in figure 4. The ring of Plate 69A is a section at the level of c_1-c_2 . The profiles of Plate 69B suggest that one bell may fit over another. The outer suggests a section at the level b_1-b_2 and the inner a section at the level of d_1-d_2 . Plate 69C is probably a section e_1-e_2 . Plate 70 is a lower magnification of the cell containing the

mitochondrion in Plate 69C. This cell contains profiles of both branching and bell shaped mitochondria.

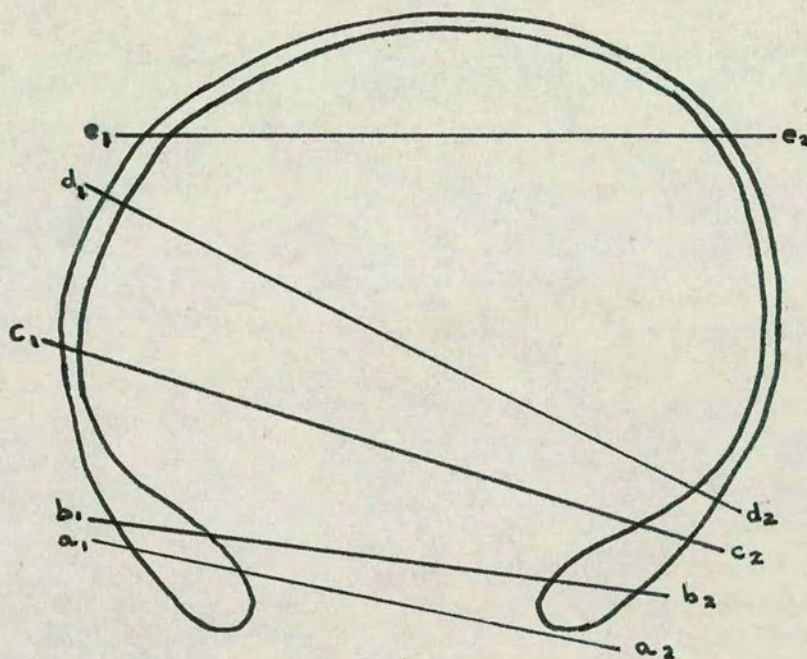


Figure 4. Bell shaped mitochondrion

3. Other organelles.

a) Crystal-containing bodies (CCBs)

After short term culture (Section IIA 1) the CCBs and the crystals within them usually appeared smaller than those in uncultured material. Some callus cells from tissue which had been cultured for 28 days contained large CCBs with large crystals (Plate 71A). These cells were vacuolated and possibly non-meristematic. Frederick et al. (1968) suggested that large CCBs constitute an inactive form of microbodies. They are predominantly found in storage cells and other metabolically less active cells.

b) Lipid bodies.

Lipid bodies (Plates 60, 71B and C) were found in all ages of the cultured tissue. There appeared to be an increase during the first two or three weeks culture both in the size (up to 1.5 μ in diameter) and in the number of these bodies. Plate 71B shows three lipid bodies closely clustered. This was a fairly common feature.

c) Endoplasmic reticulum. (ER).

ER was abundant in all cells but especially in tissue which had been cultured for about a month. The ribosomes on the rough ER were commonly found clustered in spirals during the first 1-2 weeks culture. After long culture fewer ribosomes were found in the ER and clusters were seen less frequently. After about a month's culture the cytoplasm of some cells was filled with whorls of membranes of rough ER (Plate 71C). No clusters of ribosomes were found. Whaley, Kephart and Mollenhauer (1964) found that modifications of ER, including the formation of whorls, could be attributed to 'unfavourable conditions'. Whether this was the situation in the tuber cells is not known.

Membranes similar to ER were connected to what appeared to be developing vacuoles (Plate 72A). It has been suggested that vacuoles may develop from ER (eg. Bowes, 1965). However work carried out by Manton (1962) suggested that in Anthoceros there was no continuity between the stellate vacuoles and ER.

d) Dictyosomes.

The dictyosomes in the cultured tissue usually had flat cisternae and were surrounded by many vesicles (Plate 72B). However in some of the older tissue cultured for 28 days dictyosomes with curved cisternae were observed (Plate 72C). This again, like the presence of

large CCBs in these cells indicated a differentiation of the cells similar to that of the uncultured quiescent tuber cells.

DISCUSSION.

The starting point of this investigation was a study of the storage parenchyma cells of Jerusalem artichoke tubers. These cells exhibit a low metabolic rate. Under natural conditions they remain quiescent throughout the winter and in the following spring yield their storage products during the development of the new plant. The storage cells then collapse and wither away. However when pieces of tissue excised from the tubers are placed in contact with the nutrient medium a proportion of the quiescent cells are converted to highly active cells in which synthesis proceeds rapidly, and proteins and nucleic acids accumulate (Mitchell, 1967, 1968, 1969). Eventually these cells divide. The first two divisions are synchronous (Yeoman, Evans and Naik, 1966; Yeoman and Evans, 1967) but with succeeding divisions synchrony disappears and a wound cambium is formed. With further culture some of the cells differentiate, nodules form, the rate of division is reduced and a callus culture is established (Yeoman, Dyer and Robertson, 1965).

The freshly excised explants are composed of uniform quiescent cells which are specialised for storage. Their major storage material is inulin which is assumed to be in the vacuole. In addition, this investigation has indicated the storage of lipid as droplets in the cytoplasm and protein and phytoferritin in the plastids. No signs of cell division have been found in these cells. The cytoplasm forms a thin lining inside the cell wall and cytoplasmic strands never transverse the large central vacuole. The nuclei are found in the cytoplasm next to the cell wall.

At the time of excision at least 95% of the cell nuclei have a uniform 2C value of DNA (Partanen, 1959; Adamson, 1962; Naik, 1965; Mitchell, 1967). This suggests that the cells are not dividing. The ultrastructure of the cells also reflected this point. The comparatively flattened shape and peripheral position of the nucleus indicated quiescence. Nuclei in dividing vacuolated cells are commonly more spherical and held in a central position by transvacuolar cytoplasmic strands (Bailey, 1920; Sinnot and Bloch, 1940, 1941; Steward, Mapes and Smith, 1958; Jones, Hildebrandt, Riker and Wu, 1960; Israel and Steward, 1966). This condition was also found in the cultured artichoke tuber tissue.

In the freshly excised material the appearance of the nucleoli also indicated quiescence; the nucleolar particles in the tuber cells were tightly packed; the granular particles usually surrounding the central fibrous ones. This is in contrast to the arrangement in dividing cells where during prophase the nucleolar particles are reported to be less compact with the granular and fibrous regions intermingled (Lafontaine and Chouinard, 1963; Brinkley, 1965; Birnstiel, 1967). The nucleoli often contain large electron transparent areas which have been called vacuoles (Lafontaine and Chouinard, 1963; Birnstiel, 1967) and lacunae (Hyde, 1967). The dividing cells in cultured artichoke tuber tissue also showed these features.

The protein and RNA contents of the cells at the time of excision, like the DNA content, are uniform (Mitchell, 1968, 1969). It has been established that a multiple ribosome structure, called a polyribosome or simply a polysome, is active in protein synthesis

(Warner, Rich and Hall, 1962; Gierer, 1963). The arrangement of the ribosomes in the quiescent tuber cells was consistent with a low rate of protein synthesis as polysomes were rarely seen. The polysomes which were seen were helical and apparently free in the ground substance. Wooding(1968) has recently reported the presence of large helical aggregations of ribosomes in some companion cells in mature secondary phloem of Acer pseudoplatanus. He suggested that these aggregations might represent inactivation and storage of ribosomes in response to some environmental influence. Perhaps this is also the case in artichoke tuber cells. However most of the ribosomes in these cells were not found in clusters but scattered singly in the ground substance. Also some were attached to the endoplasmic reticulum and a few to the nuclear envelope. The small proportion of attached ribosomes and the larger proportion of free ones in these mature vacuolated tuber cells contrasts with the reports of Buvat (1958), Setterfield (1961) and Loening (1961). These authors reported increases in membrane bound ribosomes and decreases in free ribosomes as cells matured and differentiated.

The structure of the mitochondria, the dictyosomes and the crystal-containing bodies (CCB's) and the scarcity of microtubules also possibly indicate the quiescent nature of the cells.

The respiratory rate of the quiescent tuber cells is low (Yeoman, Dyer and Robertson, 1965; Robertson, 1966; Evans, 1967) but the internal structure of the mitochondria appeared quite normal although the cup-shaped nature of many of the mitochondria could be considered as a specialised feature possibly associated with the low metabolic rate. The simplest mitochondrial form in all tissues examined was a sphere or

short rod. These are therefore assumed to be the elementary units from which all other forms can be derived. In immature tubers intermediate forms between the simple mitochondrial forms and the cup-shaped mitochondria in quiescent tubers were found. The intermediate forms had invaginations in the surface which in certain cases extended into large cavities in the centre of the mitochondria. Similar structures have also been found in animal tissues (Christensen and Chapman, 1959; and Stephen and Bils, 1965). It may be suggested that at some stage of development in certain of the simple mitochondria of immature tissue, an infolding of the surface occurs. With further development this infolding becomes progressively deeper until the membranes lining the fold are separated from that of the external surface only by a thin strip of material. As a result of this sequence the cup-shaped structure of the quiescent tuber would then be produced. The evidence available suggests that normally infolding occurs only at one point in the surface of the mitochondria but profiles from quiescent tubers suggest that occasionally two cavities may be formed in the same unit. As the tuber matures and dormancy is established growth of the mitochondria appears to cease.

Mollenhauer and Morré (1966) reported that dictyosomes in Quiescent cells were sometimes composed of curved cisternae with few vesicles. The dictyosomes in the artichoke tissue were frequently curved but were associated with a number of vesicles whether these vesicles were derived from the dictyosomes before or after the low metabolic rate was established is not known and therefore their significance is uncertain. The role of dictyosomes in metabolically active cells is discussed later.

CCBs have been interpreted by Frederick et al. (1968) "as a

specialised type of microbody characteristic of metabolically less active cells." They also reported that the association between CCBs and endoplasmic reticulum in the cells was not as prominent or as frequent as the intimate association observed between microbodies and endoplasmic reticulum in metabolically active cells. The large CCBs in artichoke tuber tissue were not associated with endoplasmic reticulum and are probably characteristic of the quiescent tuber cells.

It has been suggested that microtubules may play a role in cellulose microfibril deposition since the microtubules occur in the cytoplasm adjacent to regions of actively growing walls and their orientation coincides with that of the newly deposited microfibrils (Ledbetter and Porter 1963, 1964; Hepler and Newcomb, 1964; Wooding and Northcote, 1964; Cronshaw and Bouck, 1965). Assuming that this suggestion is correct, it is not surprising that in the quiescent tuber cells there were only a few microtubules found as presumably there was hardly any cell wall deposition taking place.

As the tight clusters of plastids, which were found in the freshly excised tissue, dispersed during culture they were at one time considered to be a possible characteristic of quiescent cells. However clusters were also found in cells in the cambial region of small developing or immature tubers. These cells were apparently not quiescent but meristematic.

The clusters of plastids in the artichoke tubers were similar to those described at the level of the light microscope by O'Brien (1951) in the scutellum of germinating wheat and rye. O'Brien found that plastid development took place in clusters. The earliest recognisable stage

was the appearance of very small bodies from which the plastids developed, in an otherwise homogenous area of cytoplasm adjacent to the nucleus. After the formation of a tight cluster, probably similar to that in the artichoke, the plastids dispersed. Each plastid consisted of a head and tail, the latter often having a second small head at the other end. The dispersed plastids subsequently developed into amyloplasts.

An association of plastids has also been reported in the tapetum of Cannabis by Heslop-Harrison (1963). Here, however, wefts of endoplasmic reticulum radiating from a central island interpreted as part of the nuclear envelope were apparently connected to plastids and mitochondria. In the artichoke clusters no such central island was observed although small pieces of endoplasmic reticulum were sometimes found in the tail region. However serial sections have not been cut through the whole tail region of a cluster and therefore it is possible that the partial series which were not cut contained a small central island. No connections were found between either the endoplasmic reticulum or nuclei and the plastids. Some of the sections through the narrowest plastid tails in the centre of clusters were of similar sizes to the nuclear extensions seen in cultured material. However it was always possible to distinguish the plastids by their contents and nuclear extensions were apparently not present in clusters. Mitochondria were often found near the clusters, and were occasionally interspersed with the heads, but they were not found to connect with the plastids. The phenomenon in Cannabis tapetum is probably unrelated to that in the artichoke.

The information presented here on the structure of the centre

of the tail region of the clusters is still far from complete. The plastids are tightly clustered apparently without any connection between individual tails or any other structure, and yet, in some clusters at least, are closely associated with microtubules and endoplasmic reticulum. Also no information was obtained on the formation of the clusters in the artichoke and their fate during the sprouting of the tubers under normal conditions. In cultured explants however clusters apparently dispersed and starch was formed. This simplest hypothesis is : that the clusters are homologous with those described by O'Brien (1951); that both are stages in the production of new plastids; and that the artichoke clusters represent a stage after the plastids have been elaborated and before their dispersal.

It is unlikely that clusters exist only in two such distinct situations as the scutellum of germinating grains and the tuber of the artichoke were the only obvious common feature of the situations is association with storage or mobilisation of reserve carbohydrates. The very distinctness of these situations suggests that clusters may be a more general phenomenon.

Two distinct membrane-bound systems were recognised in artichoke tuber plastids by Gerola and Dassù (1960). They called the electron dense sac-like system the corpo opaco. In this investigation the corpo opaco was often irregular and connected to branching tubules and cisternae. The whole system has been called the central system. The apparently independent second system has been called the peripheral system. Two similar systems were also recognised in the plastids of etiolated and mature green leaves, etiolated stems and roots of the artichoke. Israel and Steward (1967) recognised two distinct systems

in the plastids of storage tissue from carrots.

The difference in appearance of the peripheral and central systems presumably relate to their different functions. The function of the peripheral system is not obvious but considering its position in the plastid it could be suggested to have a function of transport associated with movement of materials through the plastid envelope to which it is connected. O'Brien and Thimann (1967) studied the plastids of the oat coleoptile and suggested that "blebs" from the inner membrane of the plastid envelope "might perhaps reflect a micropinocytotic activity by which proteins are brought into the plastid". Sunderland and Wells (1968) studied cultures of Oxalis dispar and they followed Buttrose (1960) in suggesting that invaginations of the inner membrane of the envelope may be involved in the synthesis of starch. Neither of these suggestions seems appropriate for the elaborate peripheral system found in some of the plastids of quiescent artichoke tubers, where the system consists of more than "blebs" and where there is no starch.

The central system in the uncultured tuber, the etiolated leaves and stems, and adventitious roots is likely to be a store of protein, whereas the thylakoids in chloroplasts in mature green leaves are presumably photosynthetic.

The ultrastructure of the freshly excised mature tuber cells has now been discussed and where possible this has been done in relation to previous knowledge on the physiological and histochemical state of the tissue. In an investigation of this type it is desirable to supplement qualitative observations, as described here, with quantitative data. However no counts on the number of profiles of organelles were recorded in this study owing to technical difficulties.

Counts could not be carried out on a per cell basis as the cells were so large that a complete outline was rarely obtained in freshly excised tissue and during short term culture, because of the grid bars on the copper grids. Also counts could not be made on an area of cytoplasm basis because of the loss of the tonoplast and the spreading of the cytoplasm in many cells or parts of cells. Counts could not be made on a per grid square basis because of the variation in the preservation of cell shape. Frequently the cell walls collapsed to some extent.

It is therefore not known if there was any significant change in the number of profiles of any of the organelles during the induction period. An increase in the number of profiles does not necessarily mean an increase in the number of organelles. This is particularly true of mitochondria from material such as cultured artichoke tuber tissue. In this the mitochondrial shape was frequently highly complex and several separate profiles could be found which were parts of a single mitochondrion. However an increase in the sum of the areas of the profiles of a particular type of organelle eg. the mitochondria would represent an increase in the total volume of that organelle in the tissue.

Qualitative changes in ultrastructure were observed in nearly every organelle after the tuber tissue had been cultured for a few hours and are discussed below. The investigation was confined to the cells in the peripheral layers of the explant. Not every cell divided in this region but dividing and non-dividing cells could not be distinguished by their ultrastructure before the commencement of phragmosome formation. The changes observed before the phragmosome appeared to occur in all cells

whereas the phragmosome presumably developed only in cells beginning to divide.

The first phase of culture, during which there is no change in cell number, is called the lag phase (Yeoman and Evans, 1967). The premitotic phase included the ' G_1 ', 'S' and ' G_2 ' phases (Howard and Pelc, 1953). Although the length of this phase is dependent on the length of storage of the tubers and may vary from 1-2 days (Evans, 1967), Mitchell (1968) showed that the length of the 'S' phase of this cycle remained approximately constant (about 14 hours). He stated that "the pre-'S' period must contain the normal ' G_1 ', combined with any specific events necessary to bring the cells from the quiescent state which they maintained in the tuber to the actively dividing state that exists in the growing explant". There is no prolonged ' G_2 ' period as mitosis was found to occur almost immediately after the 'S' period (Evans, 1967; Mitchell, 1967).

Yeoman, Naik and Robertson (1968) reported that autolysis occurred at the surface of tuber explants as the result of injury imposed during their excision. The autolysate produced was assumed to activate the cells deeper in the explant and they suggested that the length of the lag phase before these cells divided was determined by the rate at which it activated the cells.

At least some of the enzymes which bring about autolysis in animal cells are contained in lysosomes (de Duve and Wattiaux, 1966). It has been suggested (Schnepf, 1964; Bouck and Cronshaw, 1965; O'Brien and Thim, 1967; Frederick et al., 1968) that CCBs in plants may be the functional equivalent of the lysosomes of animal cells. Frederick et al., suggested that in addition they may be the functional equivalent of animal microbodies. The content of the CCBs in the tubers was not characterized

but it is possible that they contained at least some of the enzymes which could bring about autolysis of the outermost cells. During the first few hours of culture (probably during the pre-'S' phase) a conspicuous change was found in the CCBs. Only small ones with small crystals were seen. Whether these developed directly from the large ones found in the quiescent cells or whether the large ones dispersed and new ones developed is not known. The change in appearance of the CCBs presumably has a functional basis and may reflect important shifts in their activities and roles in the tuber cells.

Mitchell(1968) found that protein accumulation in the cells of cultured tuber tissue occurred during the 'S' phase. Probably the polysomes found in these cells during the present investigation were associated with the synthesis of protein. However it has not been demonstrated that the spiral and helical clusters of ribosomes were in fact polysomes in the sense that the ribosomes were bound to each other by messenger RNA as shown in HeLa cells by Penman, Scherrer, Becker and Darnell (1963). The appearances of polysomes was first observed after two hours culture, which must have been in the pre-'S' period. Nicholson and Flamm(1965) found that an increase in protein synthesis in cultures of tobacco cells was associated with an increase in the proportion of membrane-bound ribosomes which they isolated from this tissue. There appeared to be an increase in bound ribosomes in the tuber cells and a high proportion, if not all, of these were in spiral clusters. The helical clusters were free in the cytoplasm but there was no obvious increase in the density of free ribosomes. This is in contrast to the increase in density of free ribosomes found by electron

microscopy in meristematic cells from young cultures of carrot tissue (Israel and Steward, 1966; Halperin and Jensen, 1967).

Respiration of cultured tuber tissue increase steadily throughout the first 12 hours of culture (Evans, 1967). Increases in the number of cristae have been associated with increases in mitochondrial activity and the respiration rate in some plant tissues (Cherry, 1963; Simon and Chapman, 1961). However no increase in the number of cristae per mitochondrial profile was found in the tuber cells during this investigation. (See Appendix I). The mitochondrial matrix was often less electron dense with culture and in one experiment this was shown to be associated with an increase in volume of the mitochondria but the significance of this is not known. There may have been an increase in the number of mitochondria but this was not determined. Israel and Steward (1967) and Sutton-Jones and Street (1968) reported increases in the number of mitochondria during culture of carrot storage tissue and sycamore tissue respectively. However details of the counts made were not given.

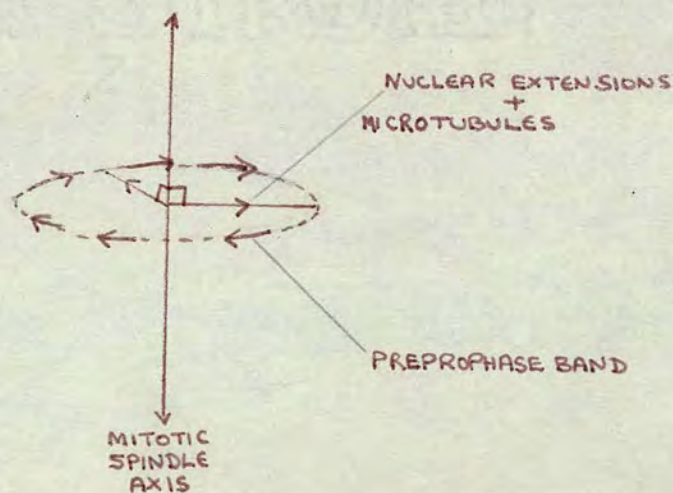
Microtubules appear to be associated with a variety of phases in the cell cycle. The significance of the observations on microtubules in the artichoke tuber cells is not completely clear but their importance in association with cell division is indicated. After about 12 hours culture (probably during the 'S' period) small groups of microtubules were frequently found near the cell wall. Whether they are associated with a renewed synthesis of wall material or with the induction of mitosis is not known. As the microtubules were seen shortly before the movement of cytoplasm and nucleus prior to cell division, they might have been

part of a preprophase band similar to that first described by Pickett-Heaps and Northcote (1966b). These authors reported that "the microtubules were orientated at right angles to the direction of the mitotic spindle and were located at the position on the mother cell wall where the future cell plate dividing the daughter cells would have joined it". In the tuber cells the numbers of microtubules recorded were never as high as those shown in the bands of Pickett-Heaps and Northcote but many of the groups were seen in a position which could have marked the future site where an anticlinal cell plate would meet the original parent wall. A high percentage of the first cell divisions were found to be anticlinal.

It is important to note that the cells in which preprophase bands have been previously reported i.e. roots and coleoptile tissue from wheat (Pickett-Heaps and Northcote, 1966 b,c); pea roots and roots of Phleum pratense (Burgess and Northcote, 1967); leaves of Nicotiniana (Cronshaw and Esau, 1968) and Chlamydomonas cells in which an analogous metaphase band has been reported (Johnson and Porter, 1968) are small and relatively non-vacuolated when compared with the cells of the artichoke tuber. Positioning of the nucleus, prior to division, in these large vacuolated tuber cells is likely to be a more complex process than that in small non-vacuolated cells. It has been suggested that the function of the preprophase band is to orientate the nucleus prior to mitosis (Burgess and Northcote, 1967). However it is possible that microtubules may need to be arranged differently to perform this function in vacuolated cells. In the vacuolated cells of the tuber, microtubules were frequently found lying parallel to one another in cytoplasmic strands stretching between the cell wall and the nucleus. These microtubules were often closely associated with

nuclear extensions. Nuclear extensions do not appear to have been described before and their significance is unknown. They consisted of tubes made of extensions of the two membranes of the nuclear envelope and contained homogenous material which was continuous with the nuclear material. The presence of nuclear extensions, their position, and association with microtubules from early prophase until the nuclear membrane breaks down suggests that they may be concerned with either the positioning of the nucleus and the cytoplasmic strands at the beginning of mitosis or the future development of the mitotic spindle. The direction of the most prominent cytoplasmic strands was nearly always similar to the direction of the majority of the cell plates at the end of the first cell division. Therefore the orientation of the nuclear extensions and associated tubules along these cytoplasmic strands or phragmosomes was apparently at right angles to the direction of the mitotic spindle. Microtubules in preprophase bands have been reported to be at right angles to the mitotic spindle axis (Pickett-Heaps and Northcote, 1966b) but they would also be at right angles to the nuclear extensions and microtubules in tuber cells (Figure 5).

FIGURE 5.



The preprophase band microtubules and the microtubules in the artichoke tuber cells have both been found in association with endoplasmic reticulum. Pickett-Heaps and Northcote (1966b) suggested that as endoplasmic reticulum is concerned with protein synthesis it might be important in microtubule synthesis. They suggested alternatively that the endoplasmic reticulum is supplying metabolites or ions and is concerned with the function of the microtubules. The observations made on the nuclear extensions and their associated microtubules and endoplasmic reticulum suggest a) that the function of the microtubules might be to orientate the nucleus prior to cell division; and b) that the microtubules are controlled, through the endoplasmic reticulum and the nuclear extensions, by the nucleus. Nuclear extensions may be characteristic of vacuolated cells in which nuclear divisions take place in nuclei suspended by cytoplasmic strands. In these cells the nuclear extensions would presumably provide the connections between the nucleus and the cell wall that are required for the correct orientation of the nucleus prior to cell division. Alternatively or perhaps in addition, the nuclear extensions and microtubules may provide support for the developing cytoplasmic strands. In the completed phragmosome this support may not be required and therefore the microtubules disappear when the nuclear extensions disappear during late prophase.

It remains to be seen whether nuclear extensions are always associated with nuclear movement during early prophase in highly vacuolated cells from other plants. Nuclear blebs, pockets, loops and other projections have been reported in a variety of immature and mature blood cells (Davies and Small (1968) give references) but these do not appear

to be related to the nuclear extensions in artichoke tuber cells or to be associated with cell division.

The formation of cytoplasmic strands and the movement of the nucleus must involve cytoplasmic streaming. Ledbetter and Porter (1963,1964) and Kane (1962) suggested that microtubules were possibly associated with cytoplasmic streaming. However since then, more detailed studies on this phenomenon have shown microfibrils (50-70 \AA in diameter), not microtubules, at the probable sites of the motive streaming force (eg. Nagai and Rebhun, 1966; Cloney, 1966; and O'Brien and Thimann, 1966). In artichoke tuber cells no such microfibrils were seen. Possibly the microtubules in the cytoplasmic strands were concerned with streaming. They were orientated parallel to the only possible direction of streaming.

Microtubules have been frequently found in the mitotic spindle (eg. Manton, 1964 a,b; Harris and Bajer, 1965; Pickett-Heaps and Northcote, 1965b,c; Burgess and Northcote, 1967). Harris and Bajer (1965) also showed that the microtubules seen in the electron microscope were equivalent to the birefringent spindle fibres seen with the light microscope.

Before the breakdown of the nuclear envelope in the tuber cells microtubules were seen around the nucleus. They appeared to be only slightly orientated and some were curved. It is probable that these microtubules would have formed the mitotic spindle later. If the suggestion that endoplasmic reticulum from nuclear extensions is associated with the forming and/or function of microtubules is correct, then it might also be suggested that endoplasmic reticulum connected with the

the outer nuclear membrane may perform the same function for future spindle microtubules.

The relationship between spindle microtubules and the chromosomes was not studied.

Ledbetter and Porter (1963) reported the presence of microtubules between daughter nuclei at telophase. Later Esau and Gill (1965) and Pickett-Heaps and Northcote (1966b,c) suggested that the microtubules found at the edge of developing cell plates probably directed the vesicles to the plate region and aligned the young cell wall in the correct position in the cytoplasm. During telophase in the tuber cells microtubules were seen at the developing edge of many of the cell plates. The absence of microtubules near other cell plates was possibly a result of variations in the quality of fixation of the cells. The cytoplasmic ground substance was more dense in the cells in which microtubules could not be detected.

The participation of dictyosome vesicles in cell plate and wall formation is well established (Whaley and Mollenhauer, 1963; Mollenhauer and Morré, 1966; Pickett-Heaps and Northcote, 1966a,b,c; Pickett-Heaps, 1967; Cronshaw and Esau, 1968). The radioautographic experiments carried out by Pickett-Heaps (1967) also suggest that the endoplasmic reticulum plays a part in cell wall synthesis. Cronshaw and Esau (1968) reported that endoplasmic reticulum may contribute some vesicles to the cell plate. In the artichoke tuber cells groups of dictyosomes were found outside the spindle region during metaphase and anaphase and near the developing cell plate during telophase. Lengths of endoplasmic reticulum were also abundant near the cell plate. The cisternae of the dictyosomes were always flat, in contrast to the curved ones in quiescent cells, and

associated with many vesicles of assorted sizes. Presumably the dictyosomes were actively involved in the synthesis of the cell plate.

Microtubules passing through the cell plate have been reported as forming plasmodesmata (Robards, 1968). This same function has been attributed to endoplasmic reticulum (eg. Frey-Wyssling and Mühlethaler, 1965, López-Sáez, Giménez-Martín and Risuño, 1965). The newly formed cell walls in cultured artichoke tissue were not found to contain many plasmodesmata and their formation was not observed. The formation of the paramural bodies associated with the new cell walls was found to be of greater interest. The membranous structures and multivesicular bodies found near developing cell plates were possibly an early stage in the development of paramural bodies. The origin of these would then be cytoplasmic and not from the plasmalemma as at this stage no recognisable plasmalemma had been formed. Multivesicular bodies are reported to develop from dictyosomes vesicles in cultured carrot cells (Halperin and Jensen, 1967) and from vesicles of endoplasmic reticulum in cotton nucellus (Jensen, 1965). This development was not observed in the tuber cells but dictyosomes and endoplasmic reticulum were both present. The significance of the paramural bodies is not known.

The microtubules found in the cytoplasm next to the plasmalemma adjacent to the new cell wall were possibly associated with deposition of cell wall material.

After the first cell division the synchrony and thus the uniformity of the tissues becomes lost. However the cells examined, during the first 2-3 weeks at least, were apparently metabolically active and could not (except in the case of developing tracheids) be

separated into different groups by the structure of their organelles. The most interesting changes during long term culture were found to occur in the plastids and the mitochondria.

In the plastids the peripheral and central systems remained distinct in the cultured explants. In the greening plastids only the central system was apparently involved in the formation of the thylakoid system. The development of the chloroplasts appeared to follow the pattern observed by Gerola and Dassù (1960) who carried out a more detailed study of greening in cultured artichoke tuber tissue. Israel and Steward (1967) reported that, during greening of cultured storage tissue from carrots, a thylakoid system developed from the prethylakoidal body analogous to the corpo opaco in the artichoke. Invaginations of the inner membrane of the plastid envelope formed during greening but in appearance they were distinctly different from the thylakoids.

The variety of complex mitochondria found in tissue cultured for 2 weeks or more appeared to fall into two groups. The first group included long cylindrical rods, branched structures and plates which contained matrix and cristae throughout. They are probably derived from simple sphere and rod-shaped mitochondria but whether they also divide up to give more spheres and rods is not known.

The second group of mitochondria consisted of plates and bell-shaped forms. These had the usual mitochondrial structure containing cristae around the rim but only a thin strip of matrix containing no cristae in the centre of the plate or dome of the bell. These mitochondria were probably derived from the cup-shaped structures which were characteristic of quiescent tissue. This could occur if the circum-

ference of the rim of the cup increased and the base of the cup enlarged, leading either to the production of a flat plate or a bell depending on the relative increases in the circumference and in the area of the central sheet.

Profiles similar to those derived from the rimmed plate and from the bell have been published by Manton (1961), Albergoni (1964) and Bell and Mühlethaler (1964). The latter authors suggested that the mitochondria giving these profiles were derived from evaginations from the surface of the nucleus. Evaginations of this kind from the nuclear membrane have not been seen in this investigation. Neither has any evidence been obtained to suggest the derivation of the mitochondrion from the endoplasmic reticulum as suggested by Albergoni (1964).

The development of the different mitochondrial forms in the cultured tuber tissue was presumably related to changing metabolic and possibly mechanical conditions in the cells. Also the development of a particular form could possibly influence the development of the cell. This is illustrated by the following example: The bell-shaped mitochondria were characteristic of relatively non-vacuolated cells and cells in which vacuolation had apparently just begun. In many micrographs small vacuoles were observed within the contour of the bell. The position of the vacuoles suggests that they were possibly being formed as a result of solutes released from the surface of the mitochondrion. These solutes which were probably components of the TCA cycle would presumably be released from both surfaces. Those released from the outer convex surface could diffuse into the body of the cytoplasm whereas those released from the inner surface could only diffuse into

the relatively confined space within the bell and could therefore establish a relatively high solute concentration in this zone. These solutes could then accumulate in a membrane bound locus if one was present. From this a vacuole would be likely to develop and hence the frequent association between vacuoles and the cavity of the bell.

In conclusion this investigation has shown that the ultra-structure of cells from tissue freshly excised from Jerusalem artichoke tubers is consistent with the fact that these cells are quiescent. When pieces of this tissue are cultured on a nutrient medium ultrastructural changes are associated with the increase in metabolic activity during the induction of cell division. However the data obtained during this period is still far from complete and many gaps lie in the sequence of events. The need for quantitative data is obvious. A more detailed study of the arrangement of microtubules and nuclear extensions in relation to the cytoplasmic streaming and movement of the nucleus before cell division is also required. Associated with this a study of cell division in other highly vacuolated cells which form a phragmosome would be of great interest.

The observations made on all the artichoke plastids described in this thesis suggest that the possession of two independent membranous systems may be a characteristic of plastids in general. The prolamellar body and the corpo opaco to which it was apparently connected in the etioplasts and the stroma lamellae and grana in the chloroplasts form a central system within the plastids and the peripheral system is always distinct, though the amount of it varies with the type of plastid. Many published micrographs from other laboratories appear to show two independent membranous systems. A detailed study of membranous systems

within plastids, from all types of plant tissue and fixed with a variety of fixatives, would indicate whether this interpretation is correct.

It would also be interesting to correlate data on the mitochondrial activities and the respiration rates with the various mitochondrial profiles in this tissue.

SUMMARY

Uncultured material.

- 1 The explants consisted of large quiescent storage cells. These cells were highly vacuolated and the organelles were embedded in a thin layer of peripheral cytoplasm.
- 2 The nuclei lay close to the cell walls and contained compact nucleoli.
- 3 The plastids were frequently grouped in tight clusters. Two distinct membranous systems (central and peripheral), a tubular system and phyto-ferritin were found within the plastids. At the light microscope level iron and protein were located in the plastids.
- 4 Many of the mitochondria were cup-shaped.
- 5 Several of the dictyosomes had curved cisternae. The dictyosomes were associated with vesicles.
- 6 Large crystal-containing bodies, lipid bodies, smooth and rough endoplasmic reticulum, free ribosomes, paramural bodies and a few microtubules were also found.
- 7 The tonoplast was rarely preserved intact.
- 8 The ultrastructure of unsprouted stored tubers was similar to that of the freshly dug tubers. However changes were found in the plastids in sprouting stored tubers.
- 9 Observations were made on plastids and mitochondria in immature tubers.
- 10 Central and peripheral membranous systems were found in plastids in green leaves, etiolated leaves, etiolated stems and adventitious roots. The tubular system was found in all the plastids except chloroplasts.

Cultured tuber cells.

A Short term culture.

- 11 The outermost cells of the explants were cut and damaged during excision from the tuber. These cells lost their contents during culture and the cell walls collapsed.
- 12 The synchronous first cell division was preceded by a lag phase during which several structural changes were observed in the cells.
- 13 Spiral and helical polysomes formed and there was an increase in the number of ribosomes bound to endoplasmic reticulum.
- 14 Unidentified material was frequently found in the vacuole.
- 15 Starch granules formed and phytoferritin dispersed in the vacuole.
- 16 Acid phosphatase was localised in the dictyosomes which were all composed of flat cisternae and numerous vesicles.
- 17 Cup-shaped mitochondria were rarely found. The mitochondrial matrix was less dense.
- 18 The crystal-containing bodies were small.
- 19 The number of microtubules increased, firstly near the ^{PLASMALEMMMA}~~cytoplasm~~ and secondly deeper in the cytoplasm.
- 20 At the beginning of mitosis the nucleus was found away from the cell wall and was suspended in the vacuole by cytoplasmic strands. In the nucleus condensation of chromatin occurred and the nucleolar material became less compact. "Nuclear extensions" were found in the cytoplasmic strands before the breakdown of the nuclear envelope. The extensions were associated with endoplasmic reticulum and microtubules.
- 21 The sequence of events during mitosis followed the usual pattern.

22 Vesicles of various sizes, multivesicular bodies and myelin-like bodies were found in the cell plate.

23 A high proportion of the first divisions were anticlinal.

24 The clusters of plastids dispersed during the first cell division.

B Long term culture.

25 Cell size was considerably reduced during culture.

26 Tracheids differentiated after 2 weeks culture.

27 Cultures grown on the standard medium did not turn green even when illuminated.

The plastids in these cultures contained central, peripheral and tubular systems. The central system was associated with "ribs" and sometimes formed lamellae. There was no development of grana even in illuminated cultures. No crystalline prolamellar body was formed when explants were grown in complete darkness.

28 Illuminated explants turned green only when cultured on a medium without coconut milk. These cultures contained chloroplasts with a thylakoid system derived by fragmentation of the central system. A peripheral system was also found.

29 A wide variety of mitochondrial forms were found in explants after about 2 weeks culture, regardless of the conditions in which they were grown. The forms included complex bell-shapes, long cylindrical rods, branched structures and plates.

30 After about 4 weeks culture, large crystal containing bodies, large lipid bodies, whorls of endoplasmic reticulum with a few bound ribosomes and dictyosomes with curved cisternae were found in some of the cells.

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APPENDIX I

The number of cristae per mitochondrial profile and the weight of the profile (X 40,000) traced from electron micrographs of uncultured tissue and tissue cultured for 24 hours.

| Uncultured | | Cultured 24 hours | | | |
|-------------|------------------|-------------------|------------------|-------------|------------------|
| No. Cristae | Profile wt. (mg) | No. Cristae | Profile wt. (mg) | No. Cristae | Profile wt. (mg) |
| 6 | 7.0 | 6 | 16.0 | 13 | 14.6 |
| 8 | 19.3 | 7 | 24.0 | 14 | 19.8 |
| 8 | 16.3 | 8 | 22.0 | 14 | 21.2 |
| 9 | 14.7 | 8 | 13.0 | 14 | 20.6 |
| 9 | 7.0 | 8 | 15.8 | 14 | 20.8 |
| 10 | 10.6 | 8 | 15.7 | 15 | 25.0 |
| 10 | 10.5 | 9 | 14.6 | 15 | 18.3 |
| 11 | 12.0 | 9 | 22.1 | 15 | 23.3 |
| 11 | 13.8 | 9 | 20.6 | 16 | 23.7 |
| 12 | 12.0 | 9 | 25.5 | 16 | 26.9 |
| 12 | 9.5 | 10 | 22.7 | 16 | 28.8 |
| 12 | 19.8 | 10 | 24.8 | 16 | 23.7 |
| 13 | 14.0 | 11 | 19.7 | 17 | 29.0 |
| 14 | 13.6 | 11 | 22.7 | 17 | 25.1 |
| 14 | 21.7 | 11 | 16.7 | 17 | 22.2 |
| 15 | 10.9 | 11 | 16.7 | 17 | 22.5 |
| 16 | 21.4 | 11 | 22.8 | 17 | 24.1 |
| 16 | 18.1 | 12 | 26.2 | 18 | 34.9 |
| 17 | 29.8 | 12 | 16.3 | 21 | 31.3 |
| 17 | 14.0 | 12 | 19.3 | 22 | 23.7 |
| 19 | 27.7 | 13 | 24.2 | 25 | 31.5 |
| 19 | 21.1 | 13 | 18.9 | 48 | 62.0 |
| 32 | 25.6 | 13 | 31.8 | | |

| Uncultured. | | Cultured 24 hours. | |
|------------------|-----------------------|--------------------|-----------------------|
| Mean No. Cristae | Mean Profile Wt. (Mg) | Mean No. Cristae | Mean Profile Wt. (Mg) |
| 13 | 16.5 | 14 | 23.3 |

A 't' test was applied to the mean profile weights and the difference between the uncultured and cultured weights was found to be highly significant. Note: The profile weights are directly related to the area of the profile and thus to the volume of the mitochondria.

APPENDIX II

Some of the work presented in this thesis has been accepted for publication:

BAGSHAW, V., BROWN, R., and YEOMAN, M.M., 1969. Changes in the mitochondrial complex accompanying callus growth. Ann. Bot. (In press).

TULETT, A.J., BAGSHAW, V., and YEOMAN, M.M., 1969. Arrangement and structure of plastids in dormant and cultured tissue from artichoke tubers. Ann. Bot. (In press).

PLATES 1 - 72

All plates are electron micrographs except 1A & B, 11D, 12C & D, 56 and 57A & B which are light micrographs. The magnifications of electron micrographs have been corrected to the nearest 1,000.

ABBREVIATIONS IN LEGENDS

ER endoplasmic reticulum
CCB crystal-containing body
HPMA hydroxypropyl methacrylate

Electron microscopy

Fixatives

G glutaraldehyde
Os osmium tetroxide
Mn potassium permanganate

Stains

Pb lead citrate
U uranyl acetate
 KMnO_4 potassium permanganate
 $\text{Ba}(\text{MnO}_4)_2$ barium permanganate

All tissues were embedded in araldite.

eg. (G,Os,Pb)- Tissue fixed in glutaraldehyde and osmium tetroxide and stained with lead citrate.

Culture procedures

Media (Methods: Section 1B)

- standard medium--mineral salts, sucrose, coconut milk, 10^{-6}M 2.4D
- No CM--mineral salts, sucrose, 10^{-6}M 2.4D
- No CM; 10^{-5}M 2.4D -- mineral salts, sucrose, 10^{-5}M 2.4D

Techniques and conditions (Section 1C)

- stationary culture in bottles
- culture in roller tubes
- culture in conical flasks on a magnetic stirrer
- culture in petri dishes on a reciprocating shaker

eg. (standard medium:Agar:1)- Explants grown in bottles on a standard medium solidified with agar.

All cultures were grown in the dark unless otherwise stated.

ABBREVIATIONS ON PLATES

| | |
|---------------------------------|---------------------------------|
| C central system of plastid | NP nuclear pore |
| CCB crystal-containing body | NU nucleolus |
| CP cell plate | NX nuclear extension |
| CW cell wall | OD osmophilic droplet |
| D dictyosome | P plastid |
| DG dense granule | PB paramural body |
| EC encircled cytoplasm | Pd plasmodesma |
| ER endoplasmic reticulum | Pf phytoferritin |
| F fibrillar region of nucleolus | Pl plasmalemma |
| G granular region of nucleolus | PS peripheral system of plastid |
| H heterochromatin | R ribosome |
| K karyosome | S starch |
| L lipid body | T tubular system of plastid |
| M mitochondrion | To tonoplast |
| MB myelin-like body | UB unidentified body |
| Mt microtubule | V vacuole |
| MV multivesicular body | I unidentified homogenous body |
| N nucleus | II unidentified fibrillar body. |
| NE nuclear envelope | |

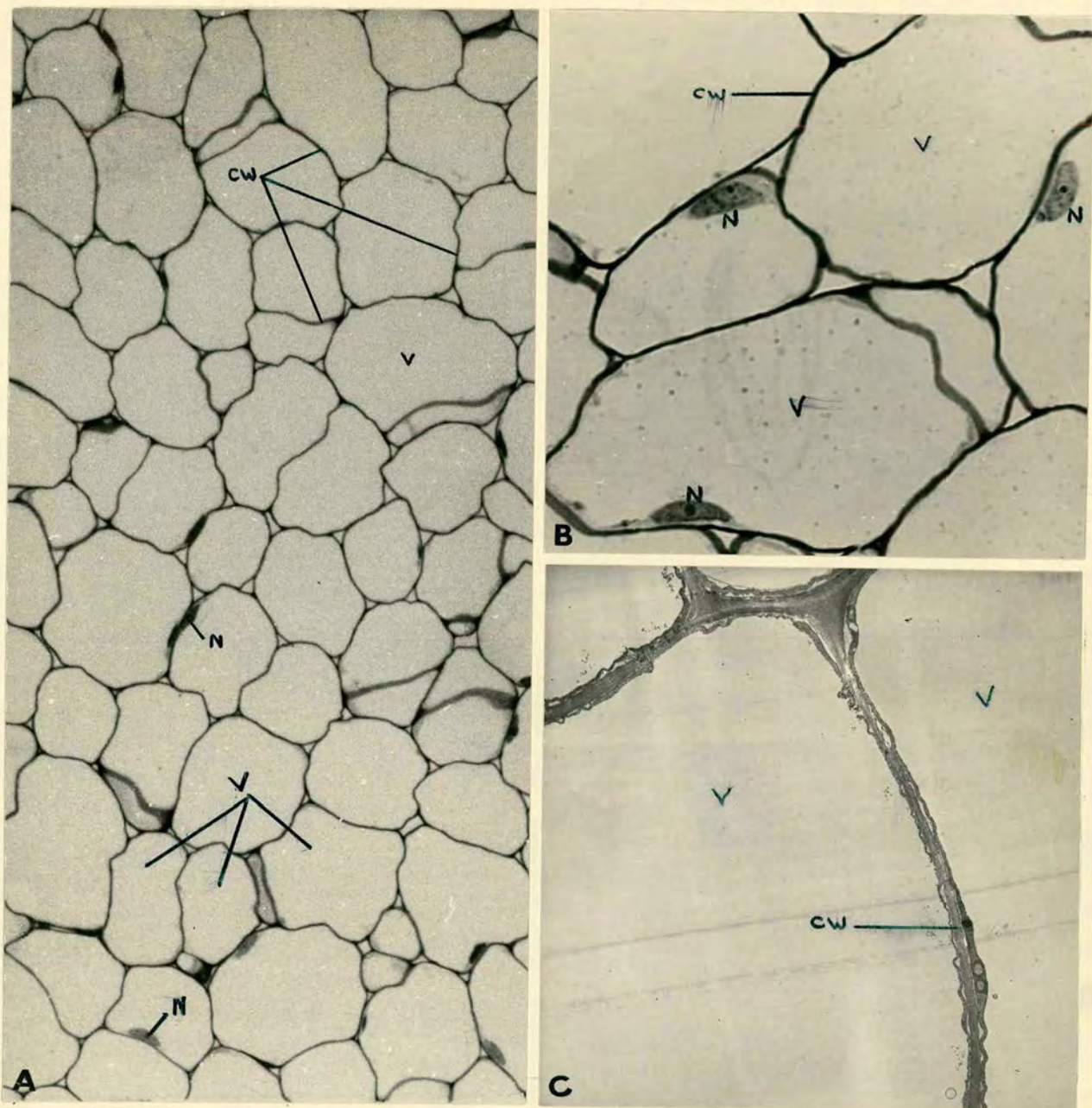


PLATE 1.

Freshly excised Jerusalem artichoke tuber tissue.

A&B Light micrographs of the highly vacuolated storage cells. The nuclei are embedded in a thin layer of peripheral cytoplasm. Fixed in acrolein, embedded in HPMA and stained with acid fuchsin and toluidine blue.

A X 580. B X 1,150.

C Electron micrograph of parts of similar tuber cells. (G,Os.Ba(MnO₄)₂) X 2,000.

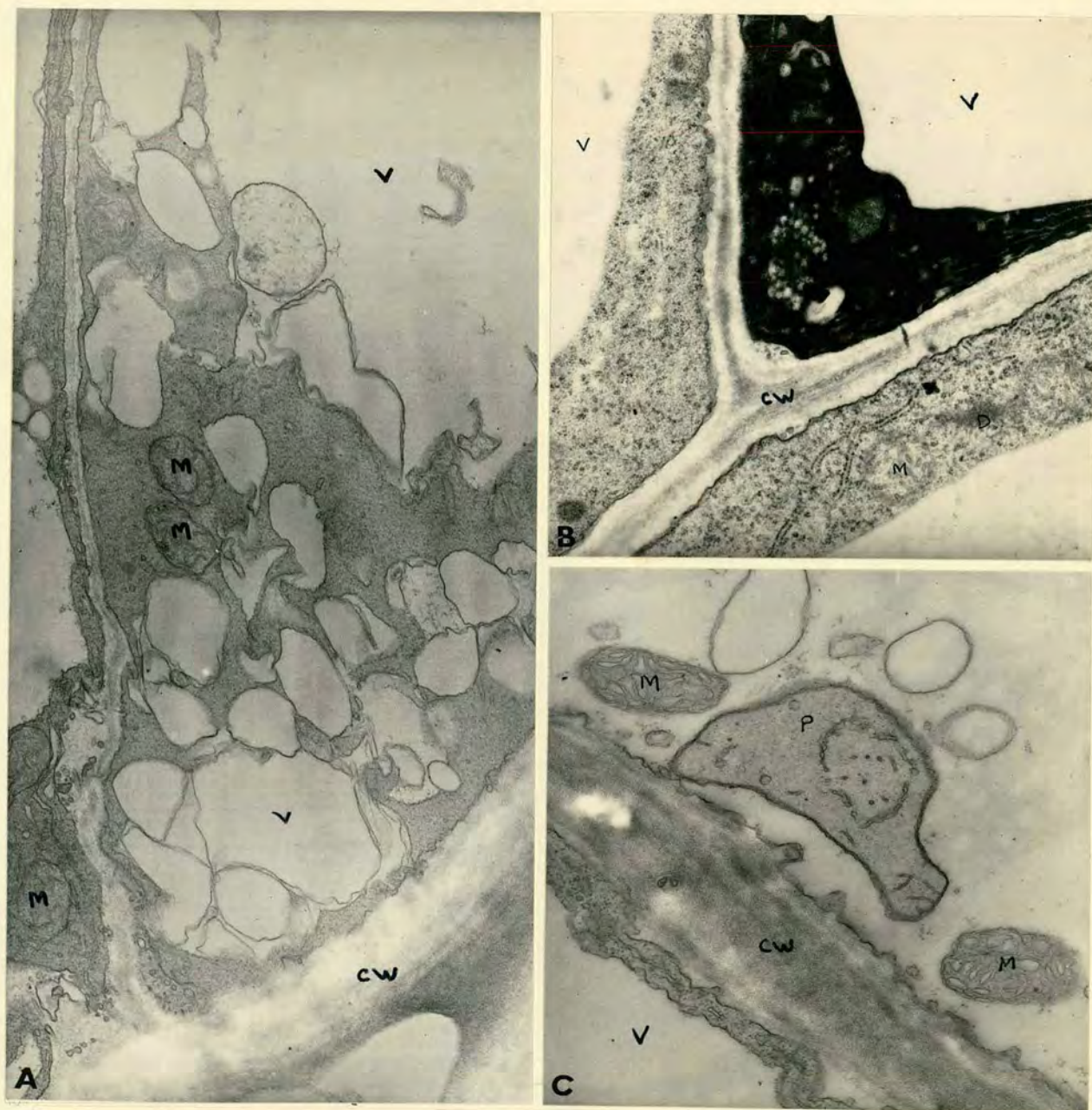


PLATE 2.

- A Freshly excised tissue. The cytoplasm in the corner of the cell contains several small vacuoles and simple mitochondria. (G,Os.Pb) X 15,000.
- B Tissue cultured for 3 days showing variations in the density of cytoplasm in different cells. (G,Os.U,Pb) X 11,000.
(By kind permission of A.J.Tulett.)
- C Freshly excised tissue. The contents of the upper cell are disorganised. (G,Os.Pb) X 21,000.

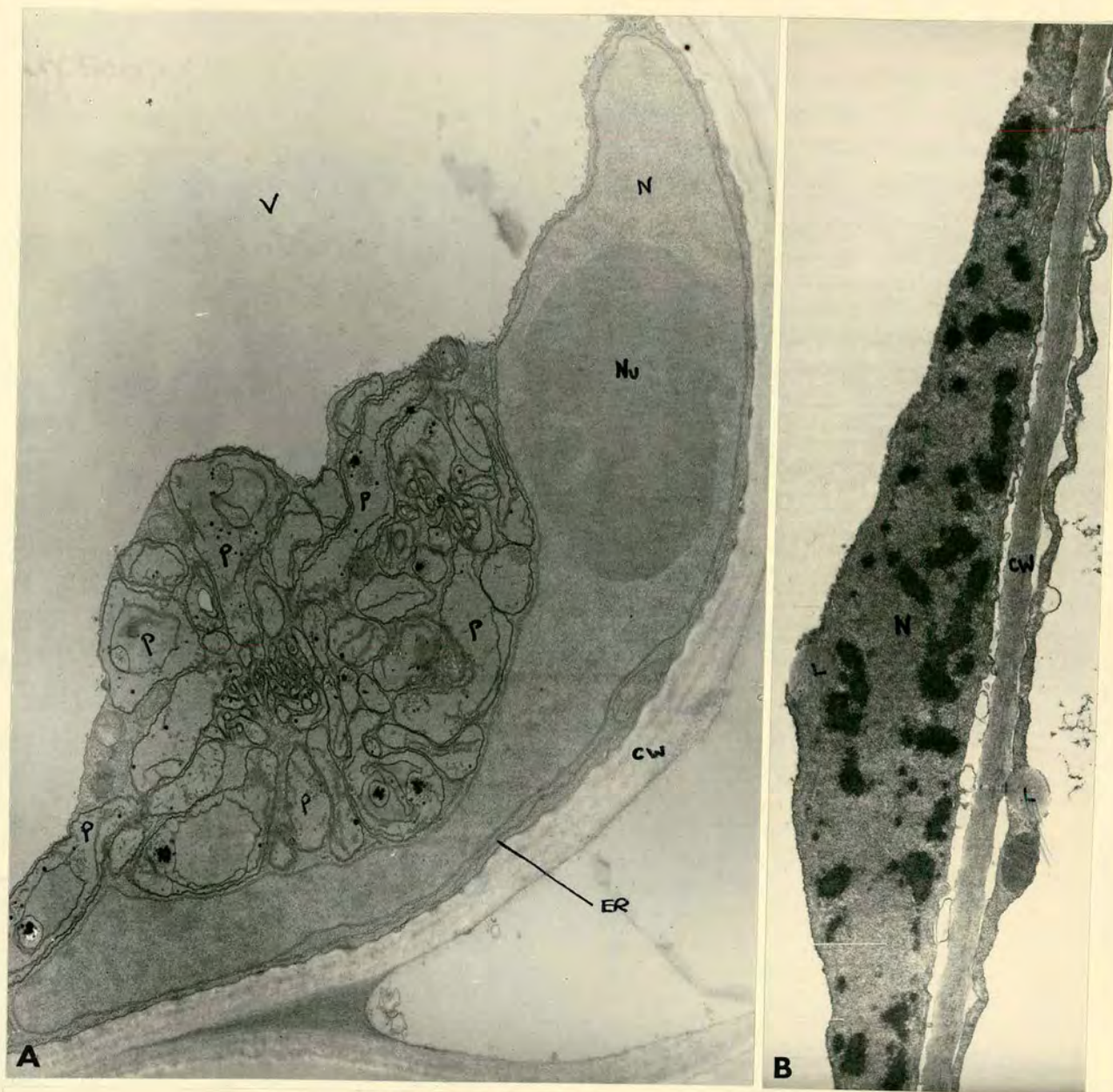


PLATE 3.

Disc shaped peripheral nuclei in freshly excised tissue.

A A large cluster of plastids with two centres of intertwined tails lies near the nucleus. A single cisternum of ER lies in the cytoplasm between the cell wall and nucleus. (G,Os.Pb) X 10,000.

B The nucleus contains dense aggregates of chromatin. (G,Os.Ba(MnO₄)₂) X 9,000.



PLATE 4.

Freshly excised tissue showing a peripherally situated, lobed nucleus containing dense aggregations of chromatin and a nucleolus with tightly packed granular and fibrillar portions. Part of a cluster of plastids lies near the nucleus. Vesicles may have been budding off the plasmalemma at the arrows. (G,Os.Pb) X 20,000.



PLATE 5.

Freshly excised tissue showing part of a lobed nucleus and a large cluster of plastids. One plastid is closely associated with nuclear lobes. Whorls of membranes resembling myelin bodies are also shown. (G,Os.Pb) X 16,000.

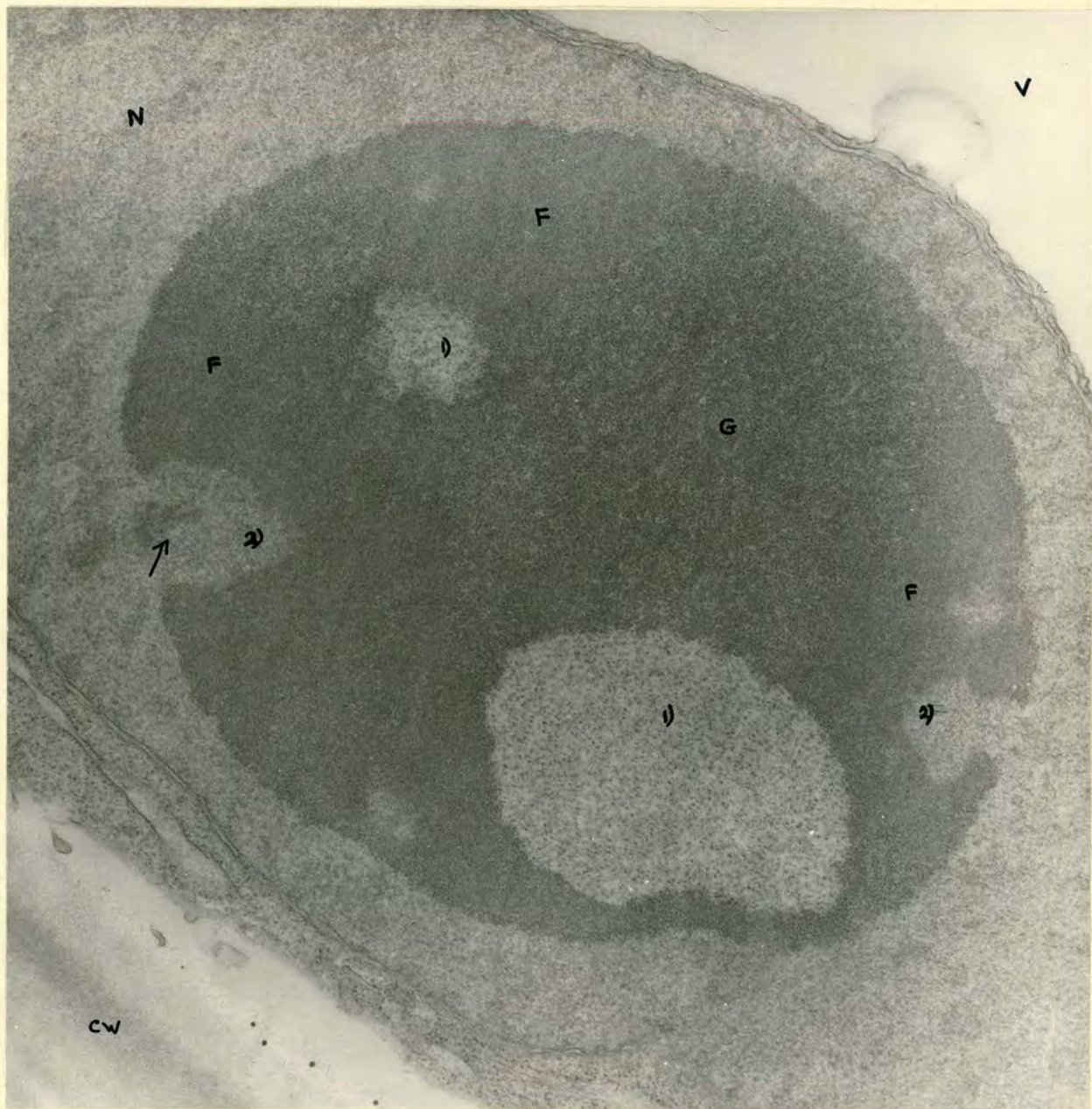


PLATE 6.

A section through a nucleolus from freshly excised tissue showing granular and fibrillar regions, and less dense areas of two types:-
 1) containing granules and fibres; 2) containing only fibres and often associated with heterochromatin (arrowed). (G,Os.Pb) X 25,000.

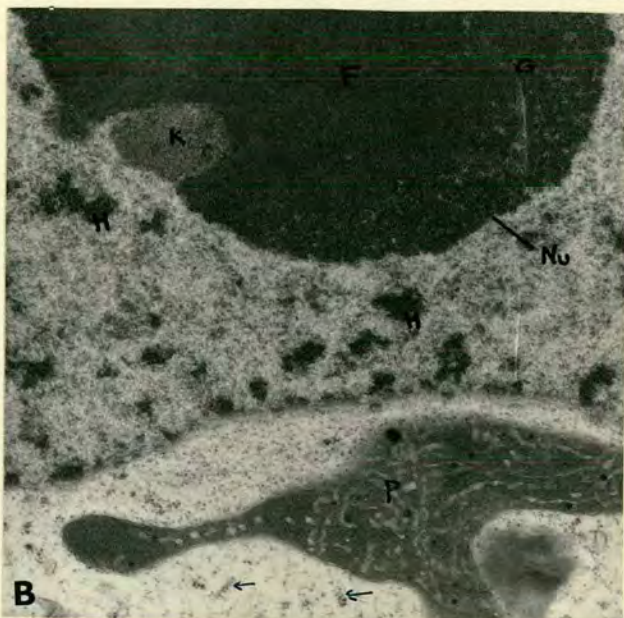
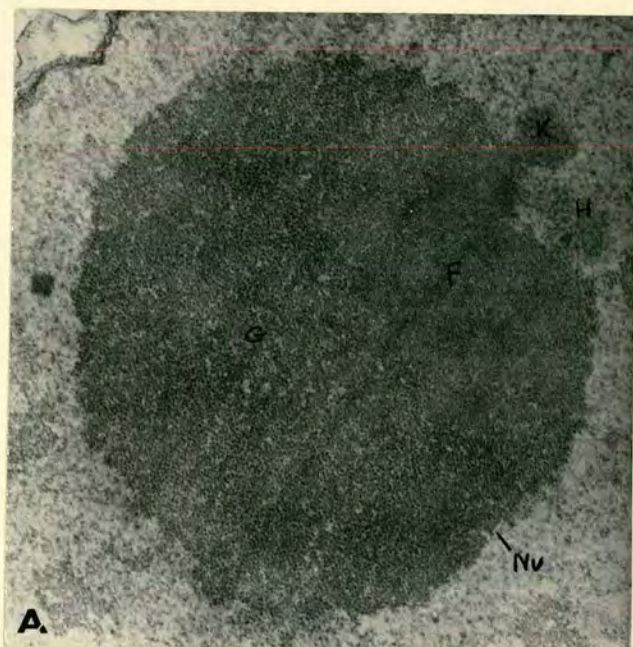


PLATE 7.

Freshly excised tissue.

A Nucleolus showing tightly packed granular and fibrillar regions and a karyosome. (G,Os.Pb) X 22,000.

B Tuber stored 1 month. The nucleolus shows a close association with a less dense body which is possibly a karyosome. Two helical ribosome clusters are arrowed. The plastid contains many profiles of peripheral system. (G,Os.Ba(MnO₄)₂) X 19,000.

C A cluster of plastids showing the large heads, containing encircled cytoplasm, surrounding the intertwined tail region. The mitochondria also contain encircled cytoplasm. The dictyosomes are curved. (G,Os.KMnO₄) X 8,000.

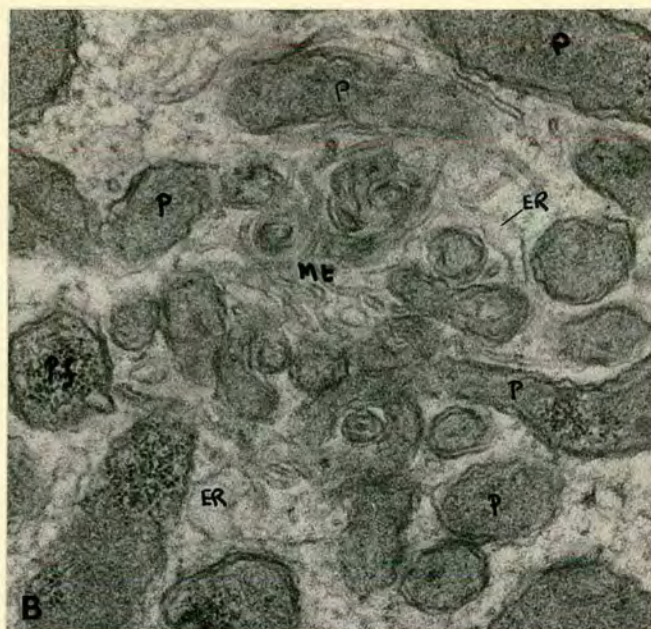


PLATE 8.

Freshly excised tissue showing the centres of clusters of plastids and their close association with ER and in B and C with microtubules.

A Wisps of central system are shown in some of the plastid tails.

The arrow marks a continuation between the peripheral system and the inner membrane of the plastid envelope. (G,Os.Pb) X 45,000.

B (G,Os.U,Pb) X 68,000.

C (G,Os.U,Pb) X 51,000.

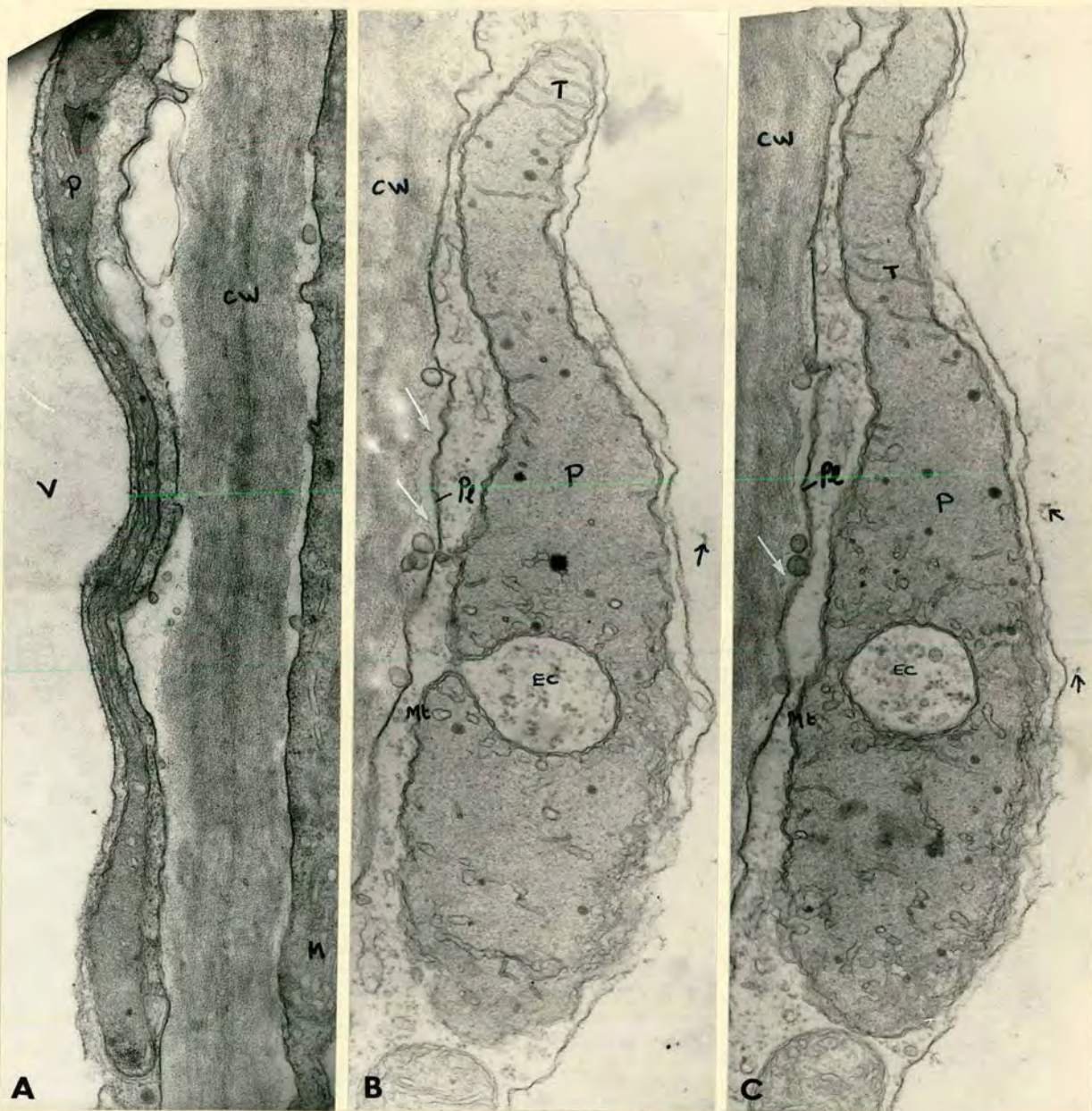


PLATE 9.

Plastids in freshly excised tissue.

A A narrow elongate plastid profile lying in a thin layer of peripheral cytoplasm. Vesicles are shown between the plasmalemma and the cell wall. (G,Os.U,Pb) X 28,000.

B&C Serial sections. The encircled cytoplasm in the plastid in C communicates with the surrounding cytoplasm in B. Both sections show microtubules near the plasmalemma, vesicles between the plasmalemma and cell wall and fibrils of cell wall material (white arrows). Scattered fibrillar material is shown in the vacuole (black arrows). (G,Os.Pb) X 29,000.

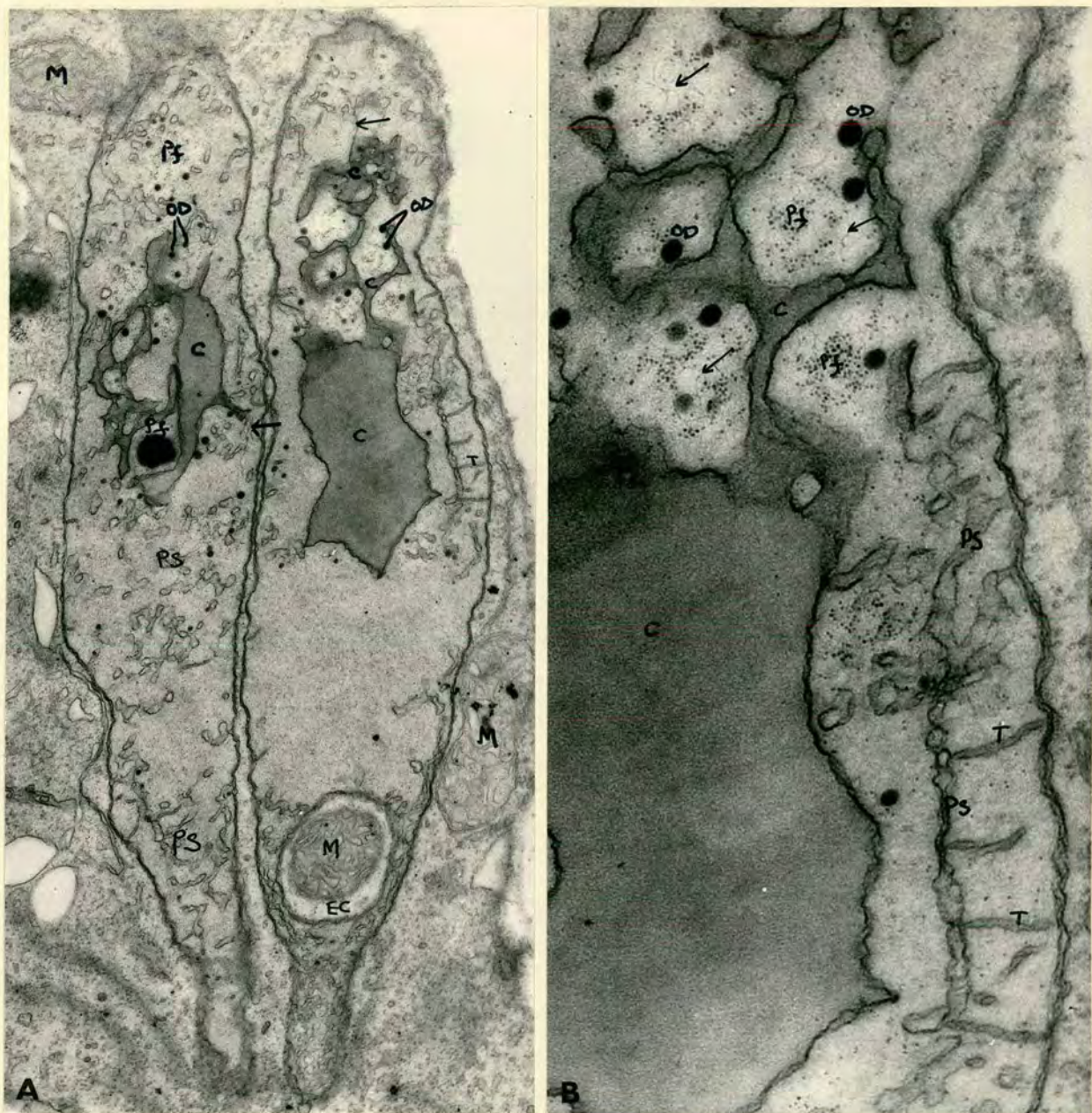


PLATE 10.

Plastids in freshly excised tissue.

- A Plastids showing central, including wisps (arrowed), peripheral and tubular systems, phytoferritin and osmiophilic droplets. A mitochondrion is shown in the encircled cytoplasm. (G,Os.Pb) X 23,000.
- B An enlargement of part of A including the characteristic plastid systems. Holes in the central system contain fine fibrils (arrowed) and loose clusters of phytoferritin granules. (G,Os.Pb) X 74,000.

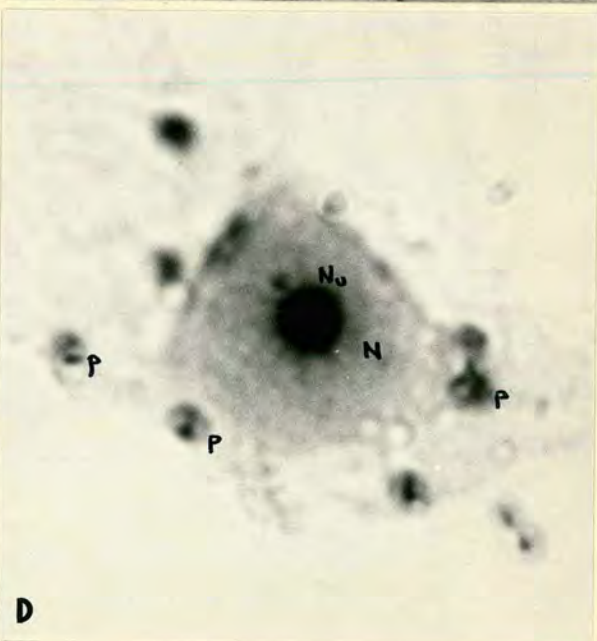
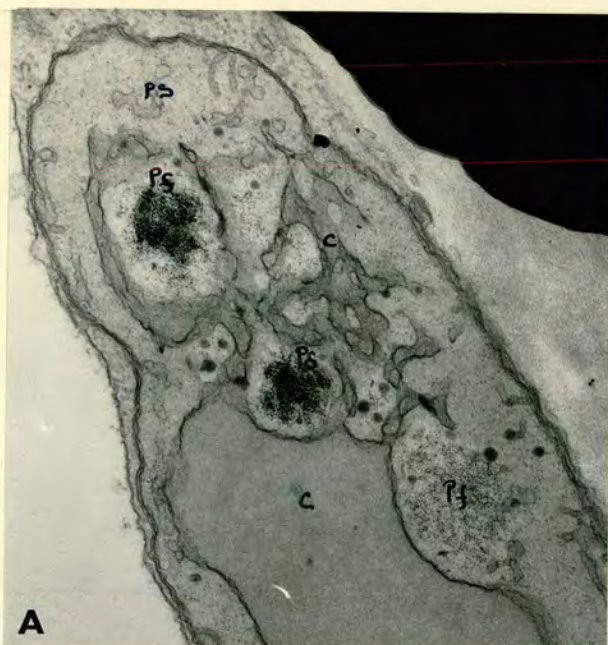


PLATE 11.

Plastids in freshly excised tissue.

A&B Show phytoferritin granules in clusters of various densities between parts of the dissected central system. (G,Os.Pb) A X 35,000. B X24,000.

C A long wisp (arrowed) is connected to the central system (G,Os.Pb) X 46,000.

D Light micrograph. Tissue stored 7 months fixed in glutaraldehyde, embedded in paraffin wax and stained for protein with mercuric bromophenol blue. The most densely stained areas are the nucleolus and parts of the plastids. X 4,000.

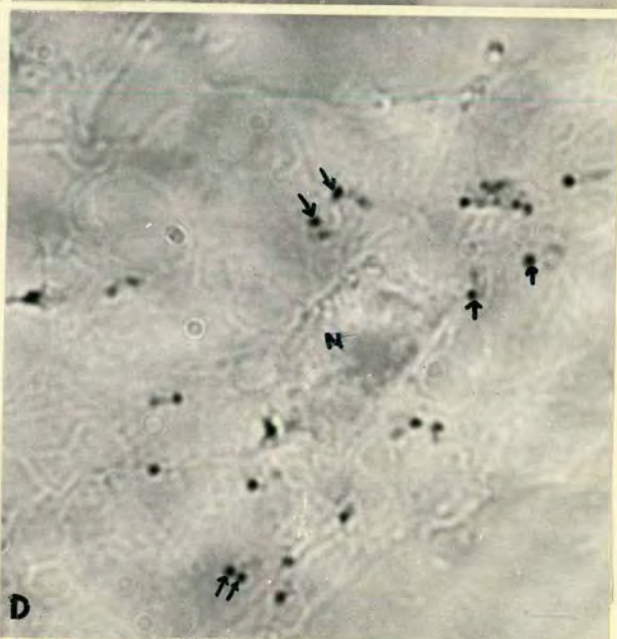
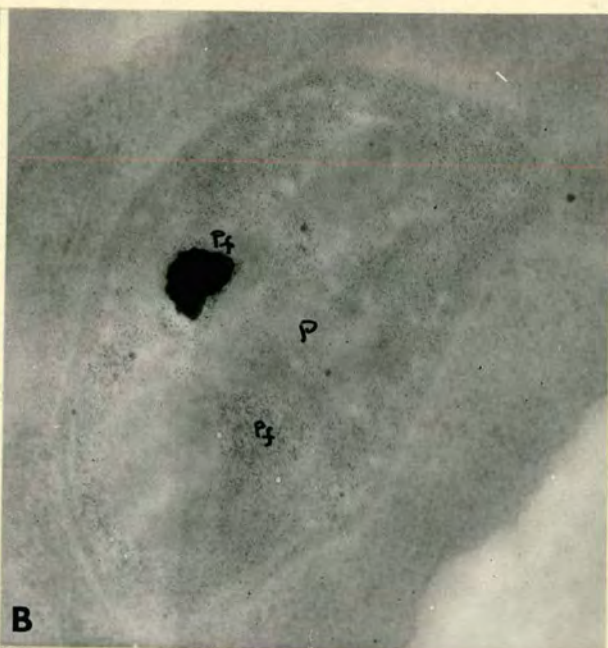
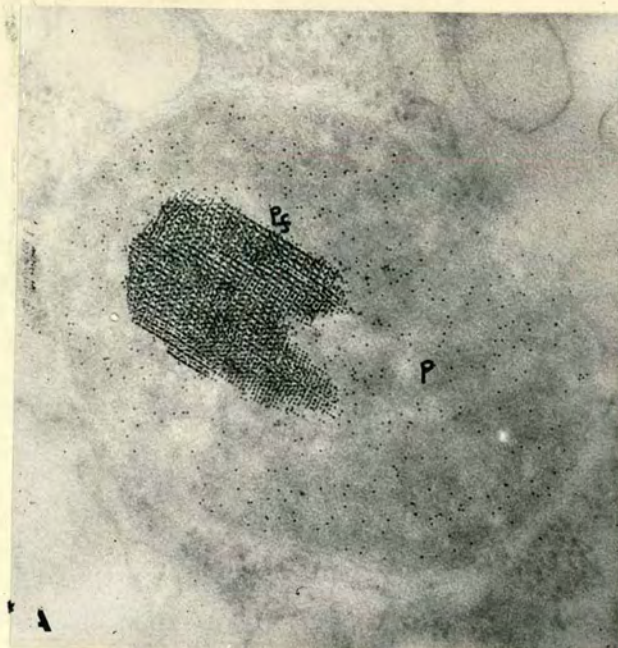


PLATE 12.

Inclusions in plastids.

A&B Tissue from immature tuber. Fixed in glutaraldehyde and osmium and treated with hydrogen peroxide. The unstained sections show electron dense granules of phytoferritin.

A Dense granules scattered and aggregated in a crystal in the plastid. X 60,000.

B Scattered and loosely clustered granules and an impenetrable mass X 36,000.

C&D Light micrographs of the same cell showing white spots (positively stained for iron) arrowed in plastids in C (bright field) appearing as black spots (arrowed) in D (phase). Tissue stored 7 months, fixed in glutaraldehyde, embedded in osmium tetroxide, and stained with lead citrate.

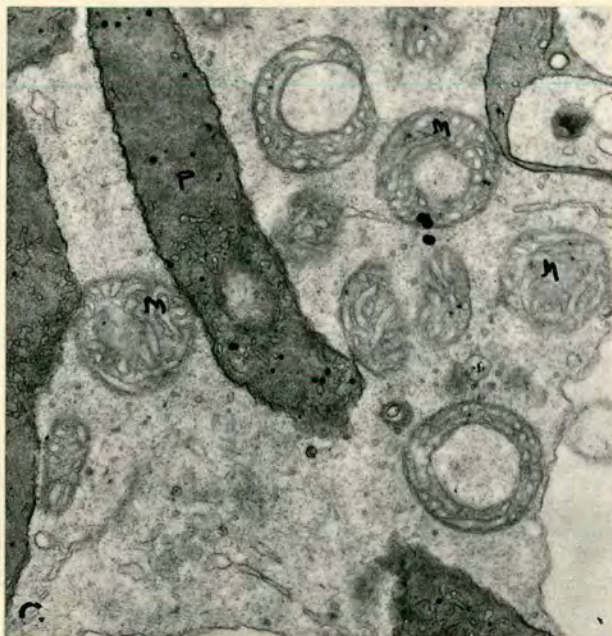


PLATE 13.

Plastids and mitochondria in freshly excised tissue.

A A phytoferritin crystal in a plastid from an immature tuber. The subparticles of each unit show a variety of geometrical patterns. (G, Os.U, Pb) X 225,000.

B Cristae forming a ring are arrowed in one mitochondrion. (G, Os. Pb) X 16,000.

C&D Sections separated by an 800A⁰ gap. Solid profiles of a mitochondrion in one micrograph appear hollow in the other. (G, Os. Pb) X 17,000.

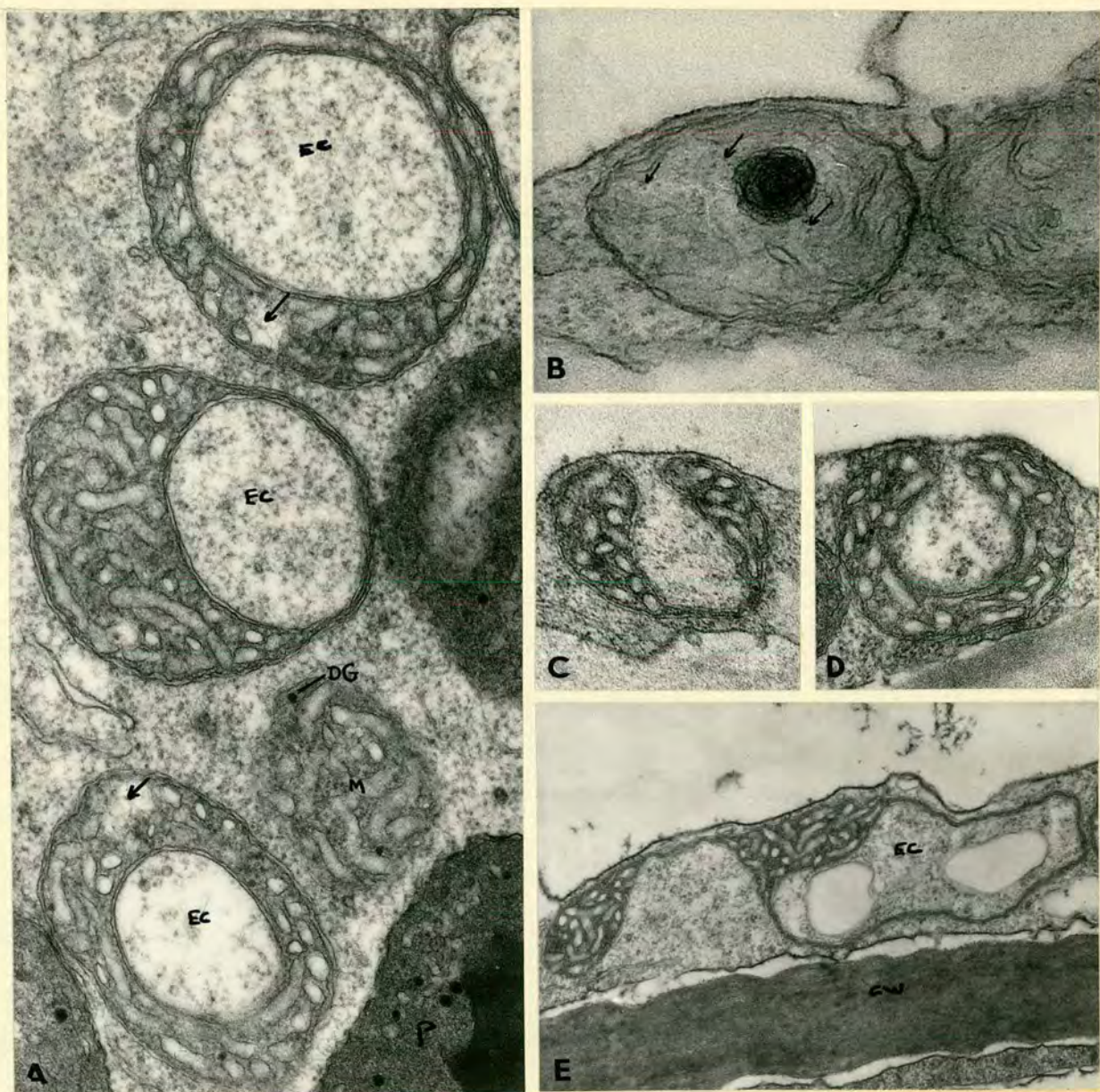


PLATE 14.

Mitochondria in freshly excised tissue.

A Three mitochondria encircle cytoplasm which is less dense than the surrounding cytoplasm. The middle one probably represents a section through $b_1 - b_2$ of Fig. 1. The other two probably represent sections through $a_1 - a_2$. The arrows mark fibres in the mitochondria.

(G, Os, $KMnO_4$) X 34,000.

B A whole of membranes is shown in the mitochondrion and small granules are arrowed (G, Os, Pb) X 63,000.

C&D U-shaped mitochondria. C contains no cristae in the basal sheet.

(G, Os, U, Pb) X 36,000. D probably represents a section through $c_1 - c_2$ of Fig. 1. (G, Os, $KMnO_4$) X 33,000.

E A mitochondrion possibly composed of two cups at right angles to each other. (G, Os, $Ba(MnO_4)_2$) X 19,000.

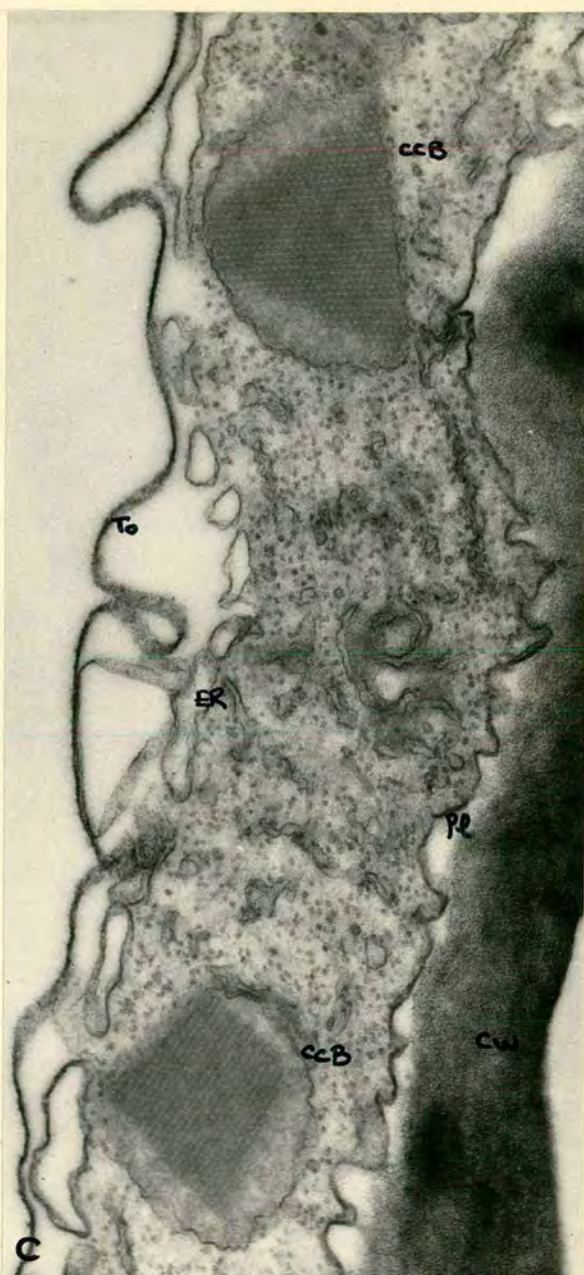
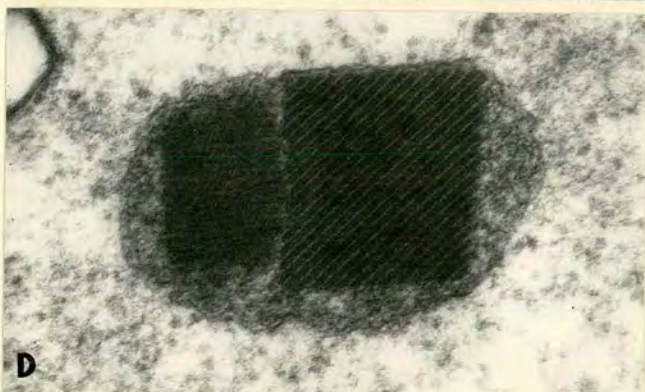
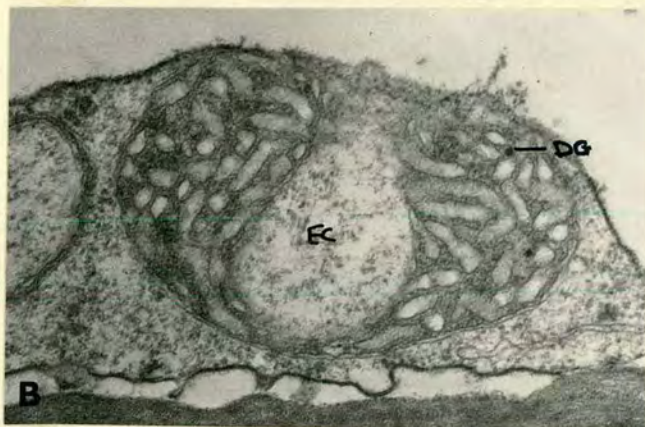


PLATE 15.

Freshly excised tissue showing the triple layered nature of the plasmalemma.

A&B Sections of the same mitochondrion separated by an 800A° gap. The encircled cytoplasm within A communicates with the surrounding cytoplasm in B. (G,Os. Ba(MnO₄)₂). X 33,000.

C Two CCBs containing large crystals. The tonoplast is partially detached and the underlying ER is apparently swollen. (G,Os.Pb) X 44,000.

D CCB containing 2 crystals showing different planes of crystallisation. (G,Os. Ba(MnO₄)₂) X 56,000

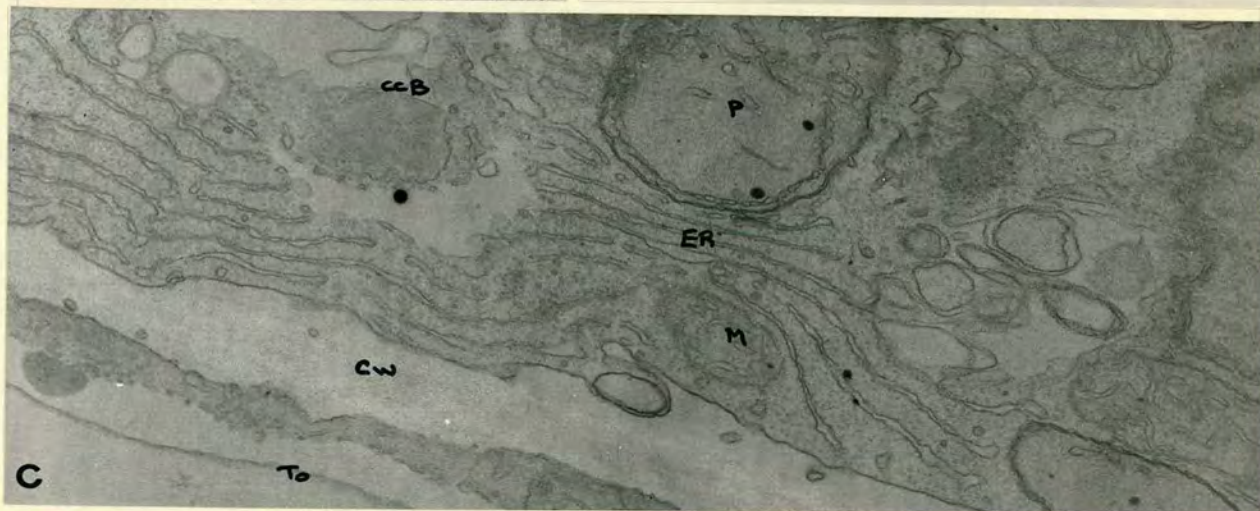
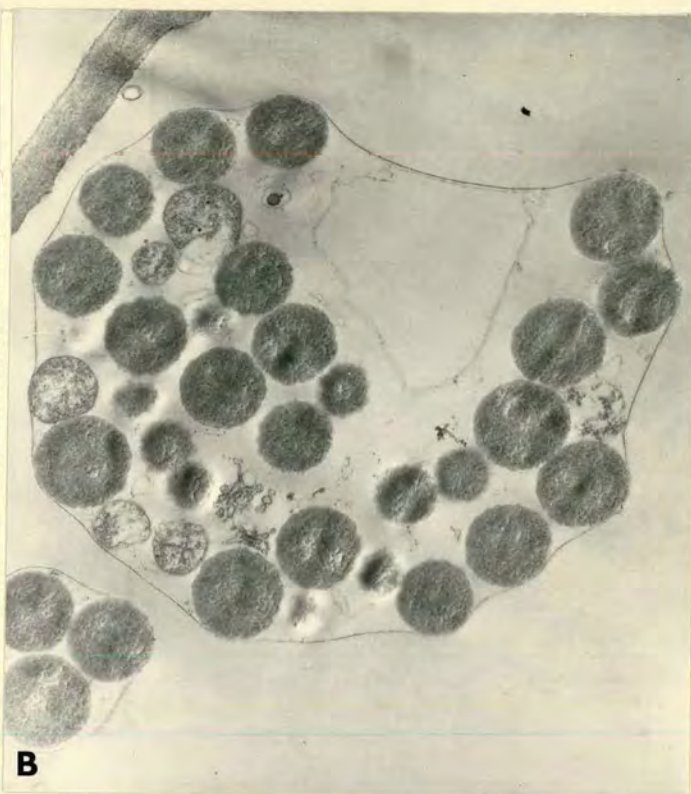
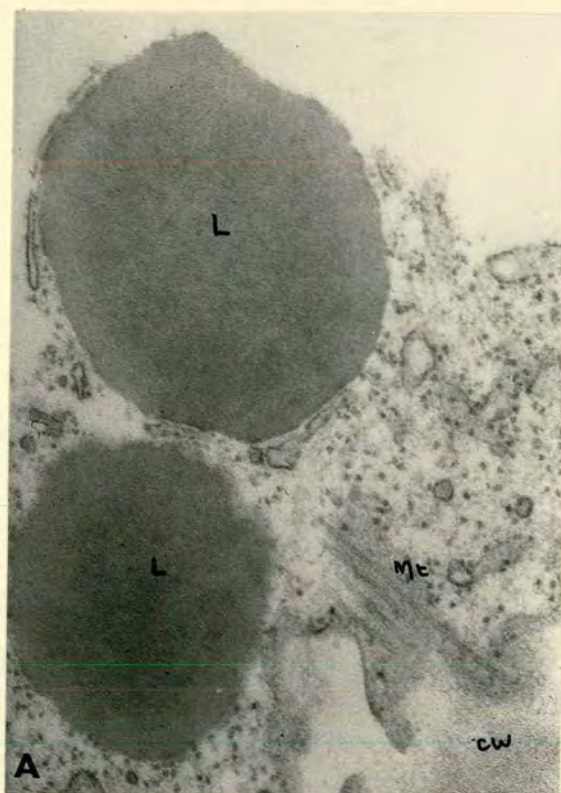


PLATE 16.

Freshly excised tissue.

A Two lipid bodies and microtubules are shown. The tonoplast is missing. (G,Os.Pb) X 41,000.

B Lipid bodies at the centrifugal pole of the cell in centrifuged tissue. (G,Os.Ba(MnO₄)₂) X 19,000.

C The ER is parallel to the cell wall. (G,Os.Pb) X 25,000.

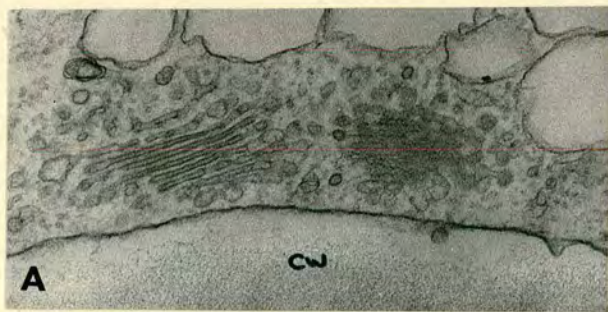


PLATE 17.

Dictyosome and paramural bodies in freshly excised tissue.

A Dictyosomes composed of straight cisternae and vesicles (G,Os.Pb)
X 42,000.

B Dictyosomes composed of curved cisternae and vesicles. (G,Os.Pb)
X 32,000.

C A paramural body containing vesicles with a triple layered structure
similar to that of the plasmalemma. An unidentified body lies in
the vacuole. (G,Os.U,Pb) X 26,000.



PLATE 18.

Paramural bodies in freshly excised tissue. (G.Os.Pb)

A A variety of vesicles and tubules are shown in the body. Small vesicles (arrowed) may be budding off or fusing with the plasma-lemma. Cell on the right: tonoplast partially detached. Cell on left: tonoplast missing. X 35,000.

B The paramural body is associated with plasmadesmata. X 36,000.

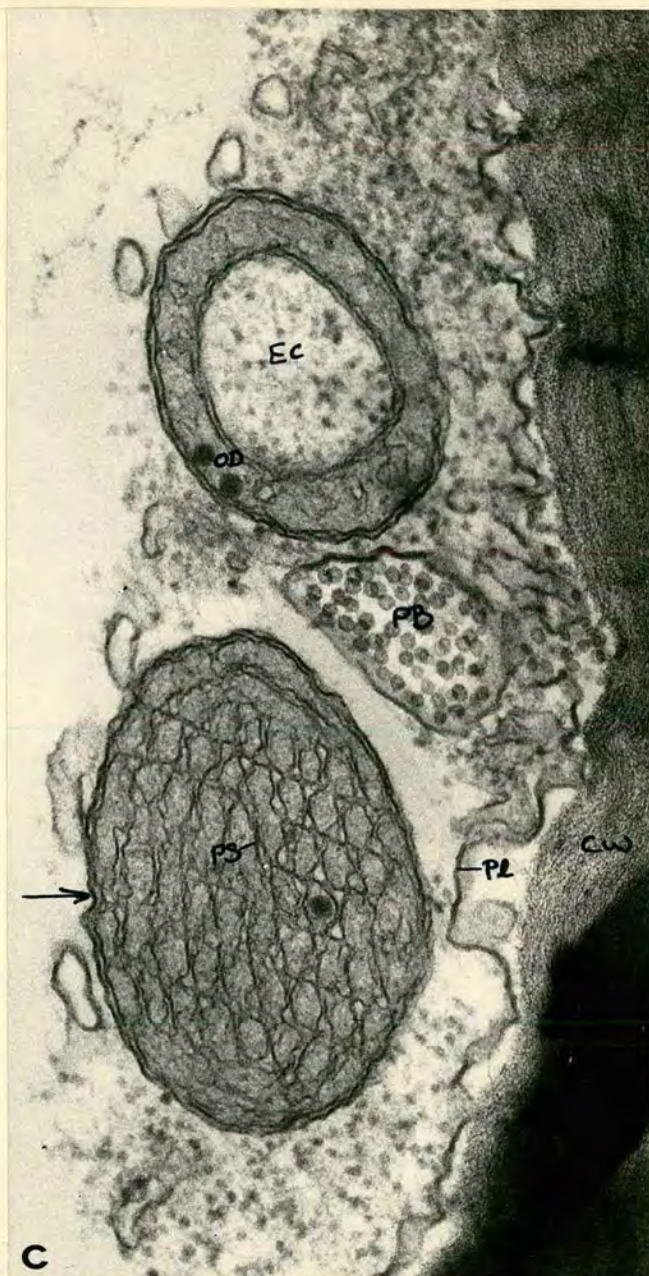
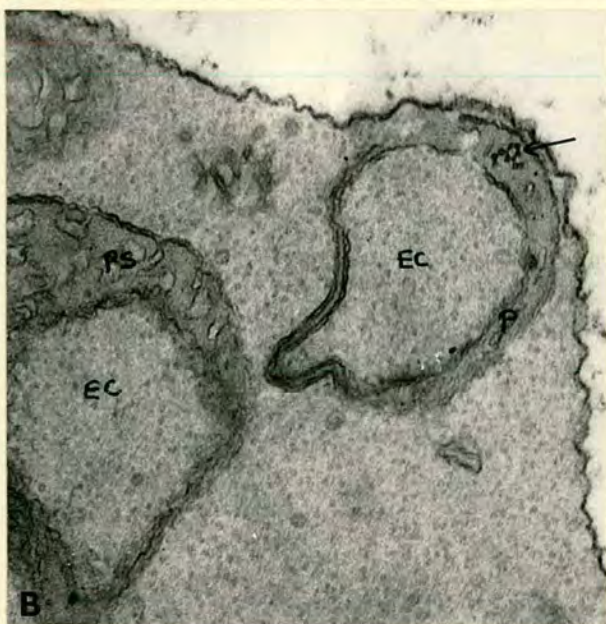
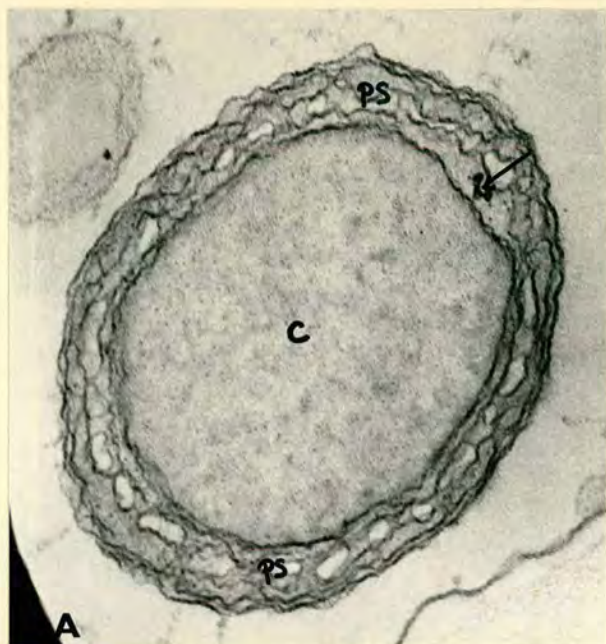


PLATE 19.

Plastids from freshly excised stored tissue. (G, Os, Pb)

A&B Stored 4 months. Small connected tubular elements (arrowed) which may be part of the central system (See Plate 26D) are shown. A also contains a regular corpus opaco and distinct peripheral system.

X 63,000. B X 51,000.

C Stored 5 months. The peripheral system in one of the plastids (arrowed) has formed a regular network. X 68,000.

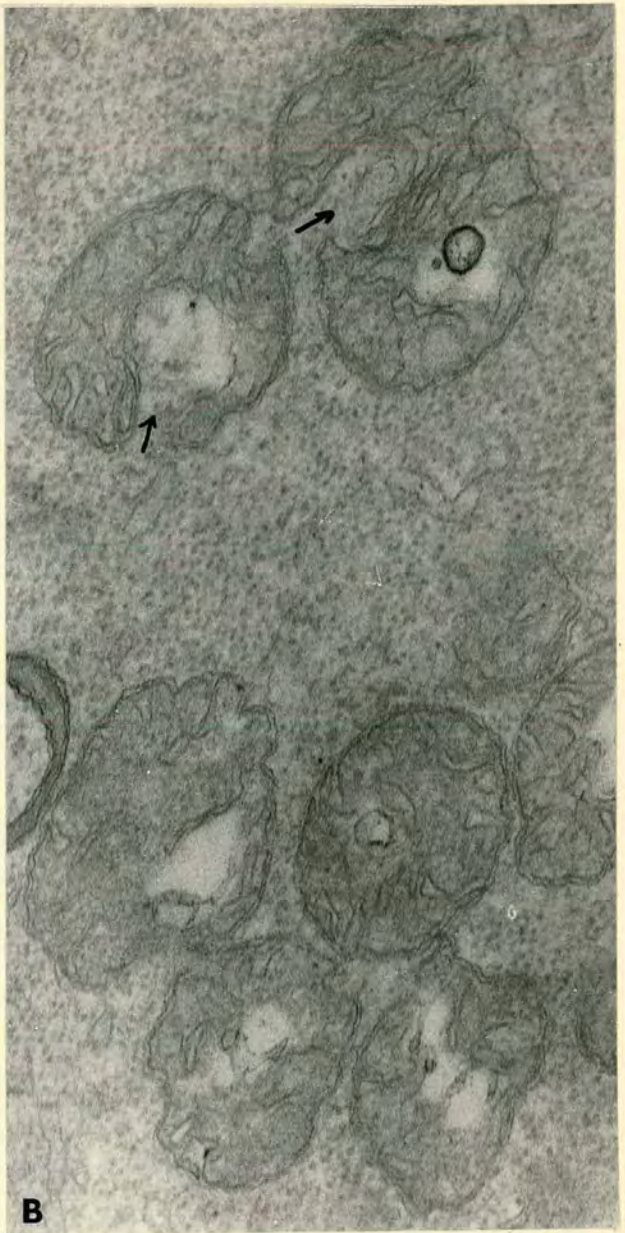
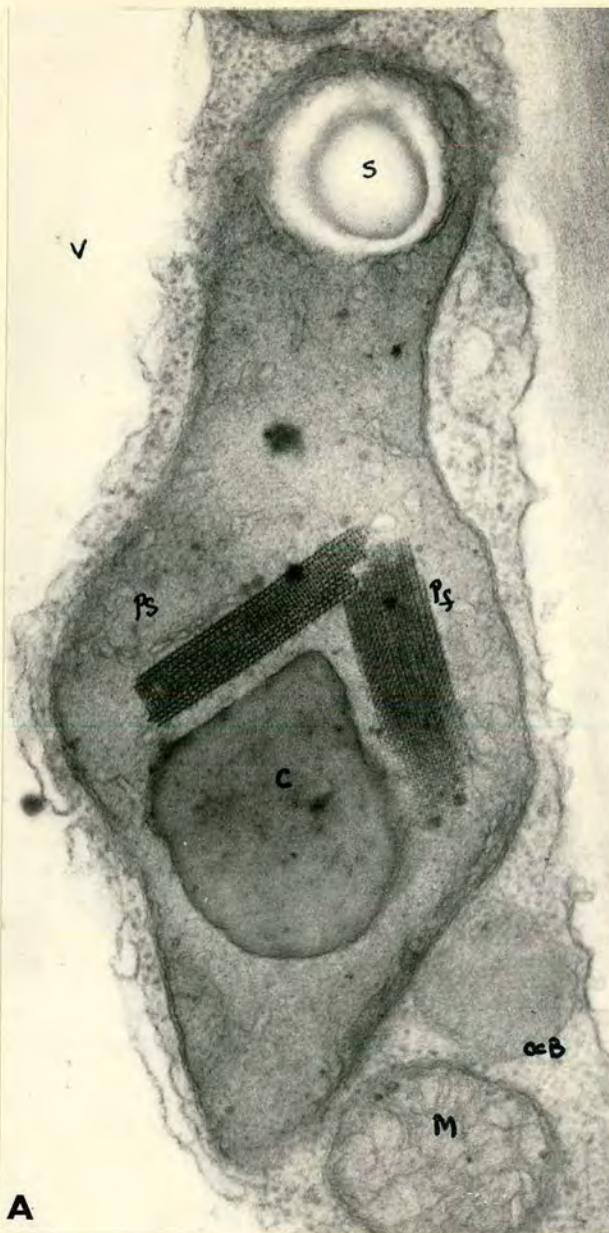


PLATE 20.

Freshly excised tissue from immature tubers (G, Os. Pb).

A A plastid showing a large starch granule and two large phytoferritin crystals. X 48,000.

B Small circular mitochondria. The arrows mark invaginations of the mitochondrial surface. X 46,000.



PLATE 21.

A chloroplast in a mesophyll cell from a green leaf. The central system is composed of grana and stroma lamellae. The peripheral system is very reduced. It is continuous with the inner membrane of the plastid envelope at the position arrowed. Starch is abundant. (G,Os,Pb) X 39,000.

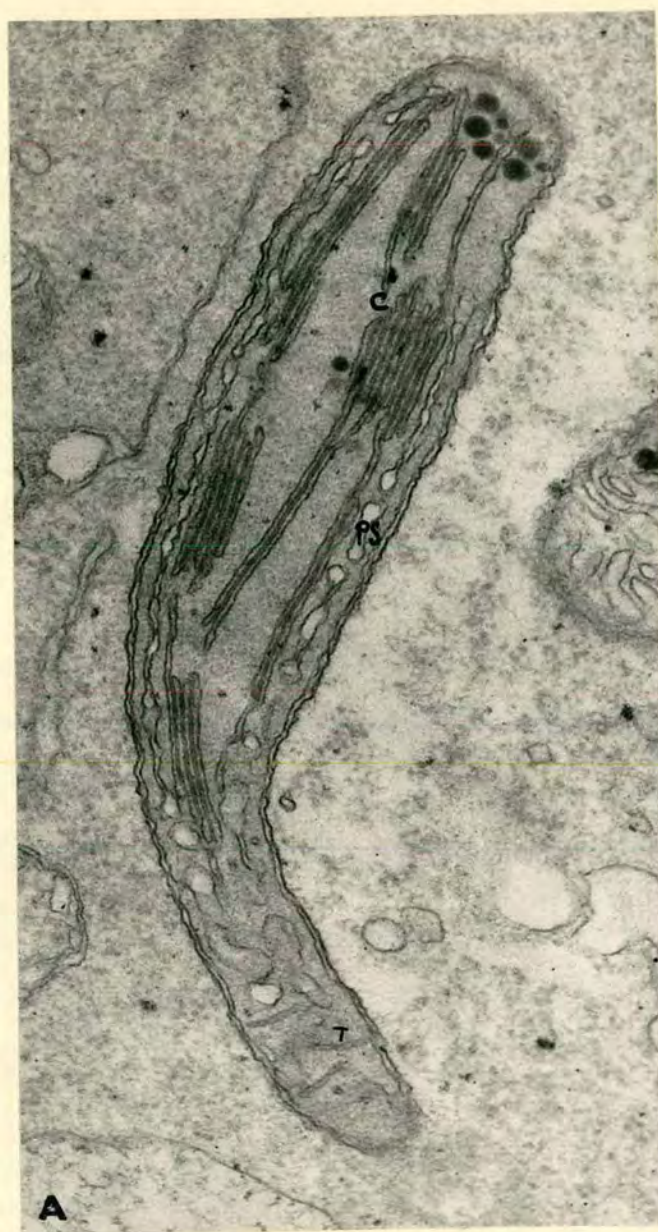


PLATE 22.

Plastids from a green leaf. (G,Os.Pb)

- A Plastid from a vascular parenchyma cell showing a less extensive thylakoid system than in Plate 21. Well developed peripheral and tubular systems are present. X 60,000.
- B Plastid from an epidermal cell. The peripheral system is again well developed and the arrow marks its continuation with the inner membrane of the plastid envelope. The grana each consist of two discs and are joined together by short stroma lamellae. X 55,000.

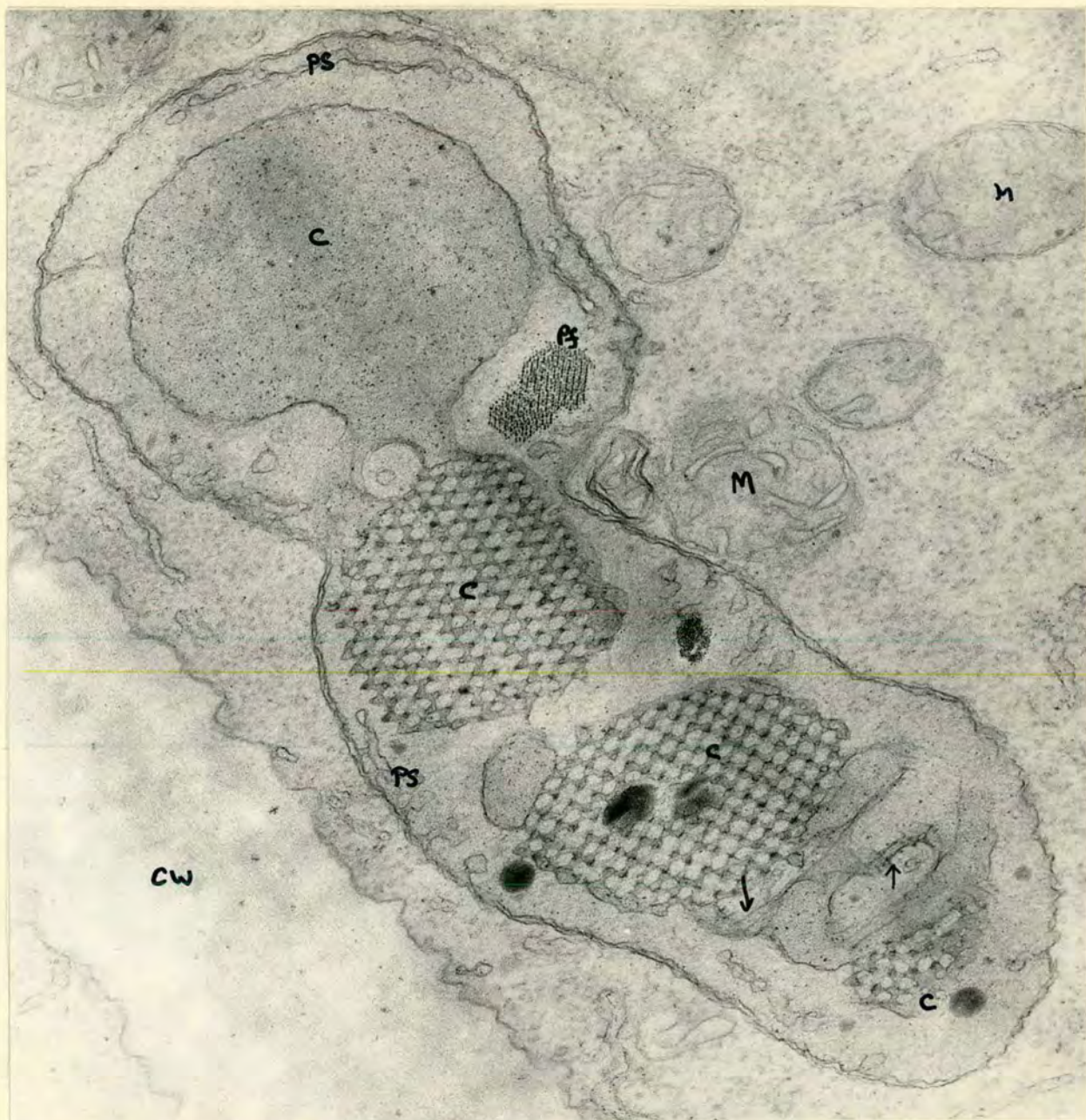


PLATE 23.

Plastid from an etiolated leaf, The central system consists of a core space and crystalline prolamellar bodies. 'Ribs' (arrowed) are associated with the central system. The peripheral system is also shown. (G,Os. Pb) X 49,000.

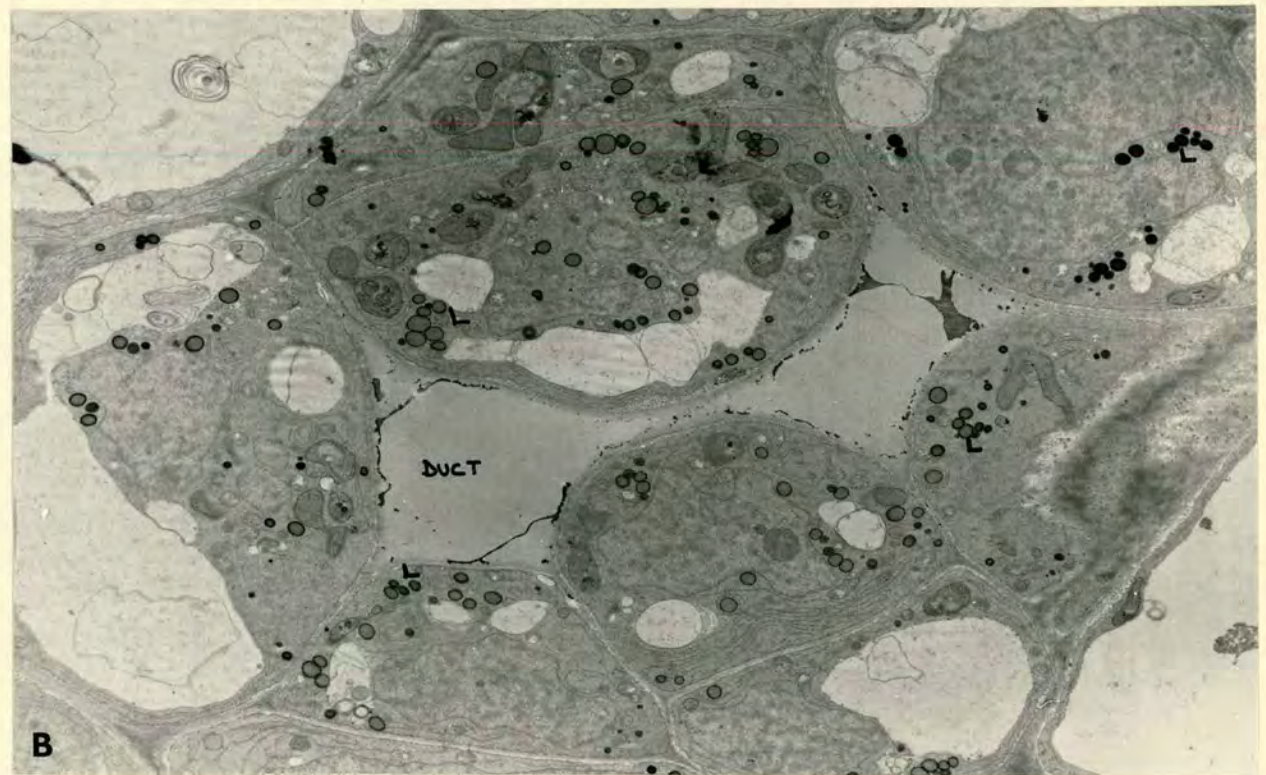
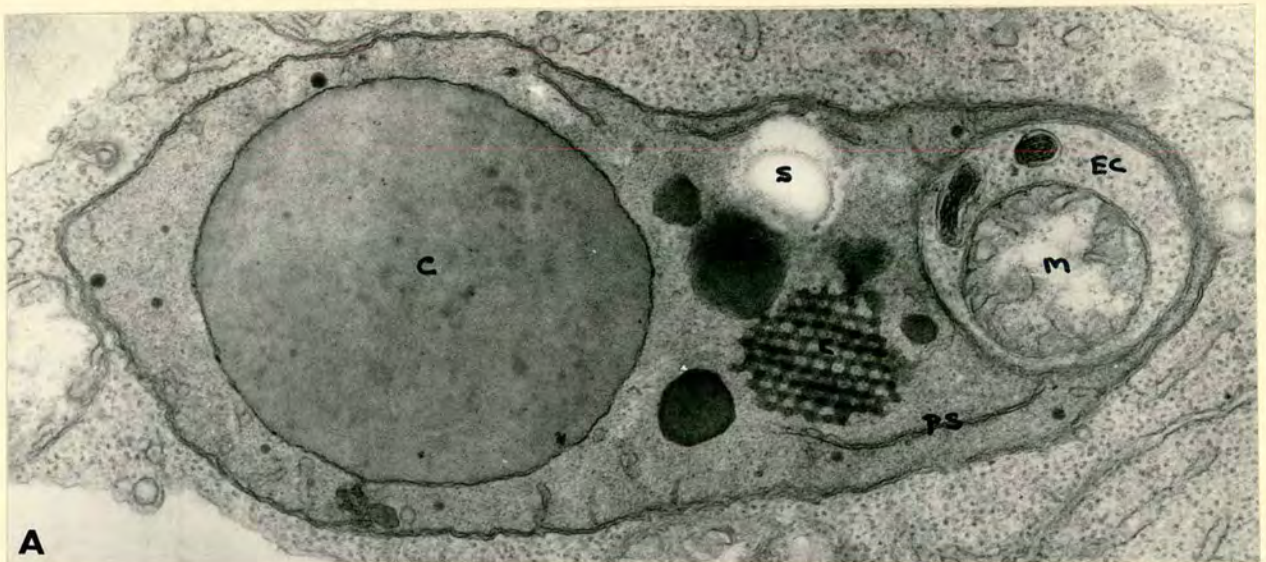


PLATE 24.

Etiolated stem tissue (G.Os.Pb)

- A Plastid showing a corpus opaco and crystalline prolamellar body. The encircled cytoplasm contains a mitochondrion and two whorls of membranes. X 39,000.
- B Lipid containing secretory cells surrounding a secretory duct. X 3,500.

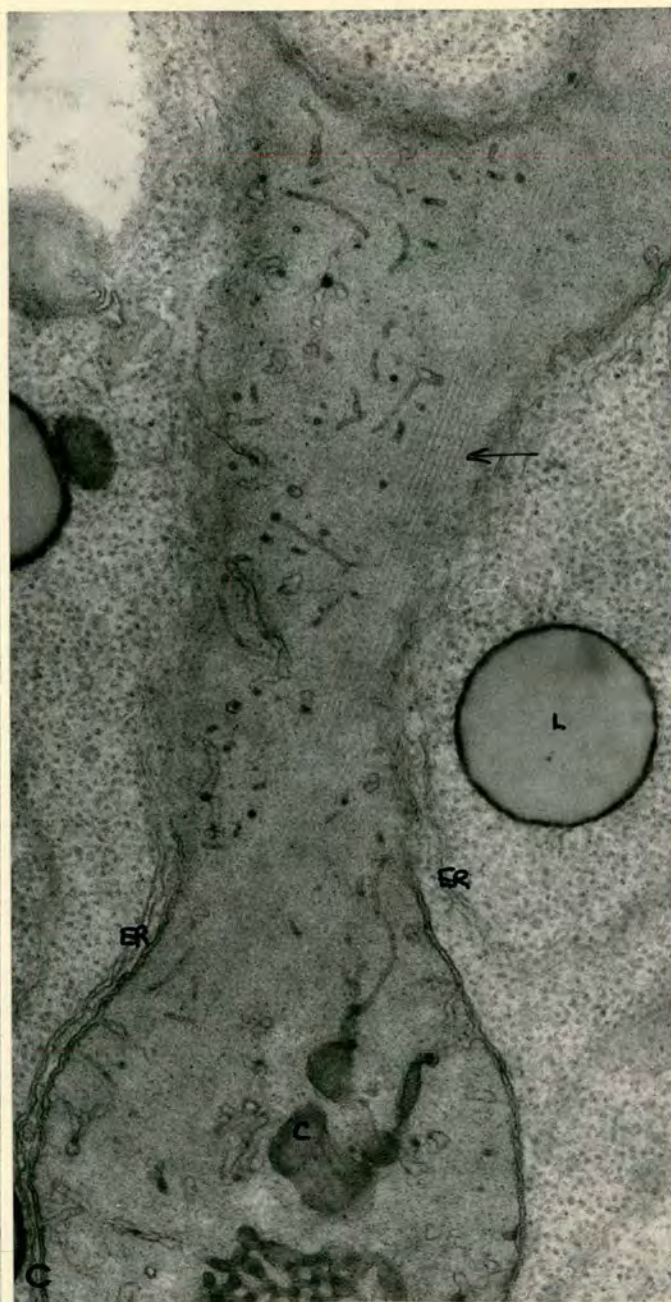
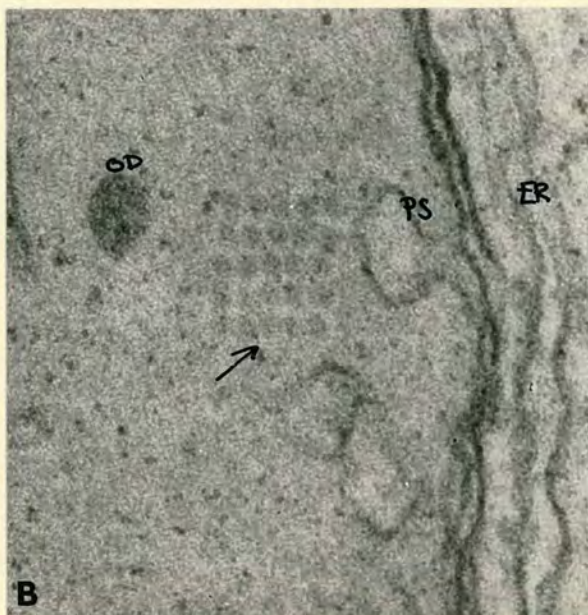
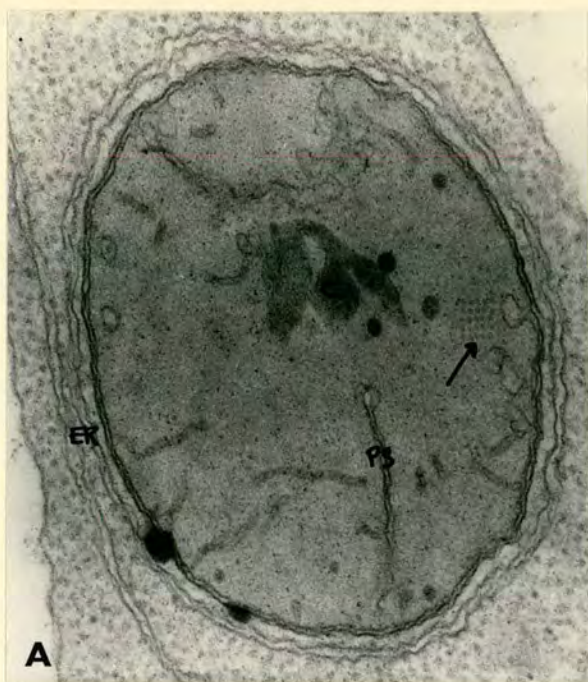


PLATE 25.

Plastids, ensheathed in ER, from secretory cells. The central, peripheral and tubular systems are shown. Fine straight filaments are also present. (G, Os. Pb)

A&B The filaments are shown in cross section. It is not clear whether they are orthogonally or hexagonally packed. A X 65,000.

B (enlargement of A) X 230,000.

C The parallel filaments are lying longitudinally in this section. X 49,000.

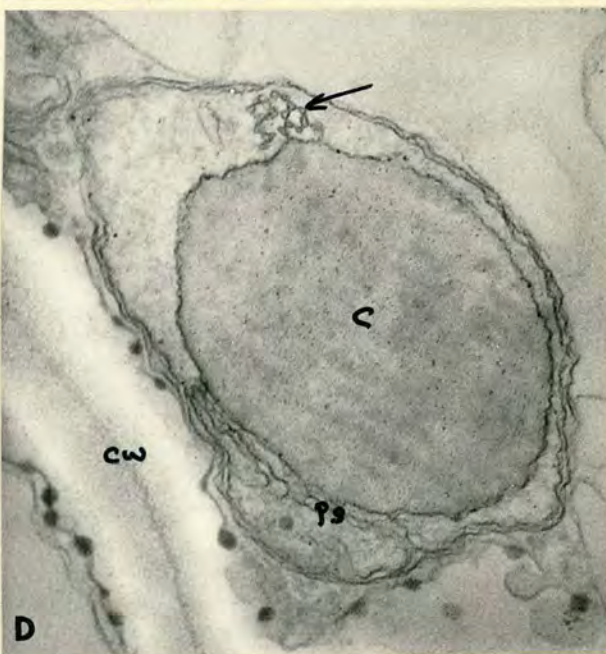
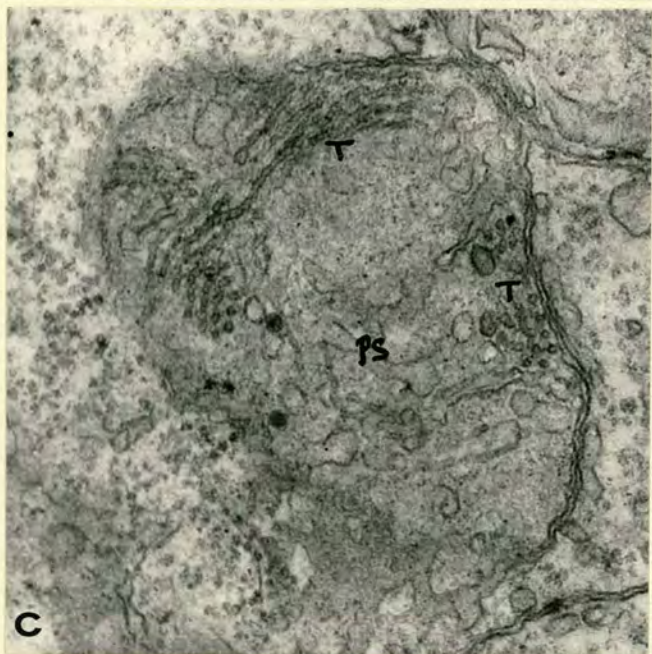
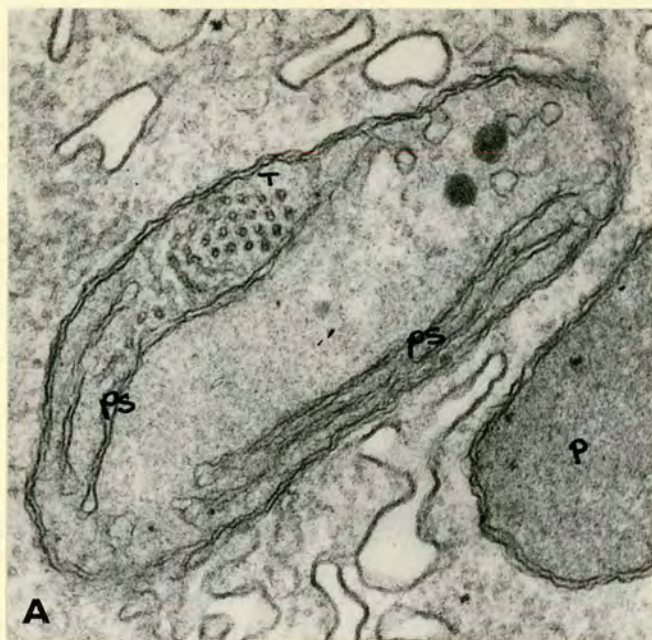


PLATE 26.

Plastids in adventitious roots sprouting from stored tubers (G,Os.Fb)
 AB&C Elaborations of the tubular system are shown. Peripheral and central
 systems are also shown. A X 70,000. B X 62,000. C X 65,000.
 D A corpo opaco associated with angular tubules is shown. (See Plates
 19A & B). X 62,000.

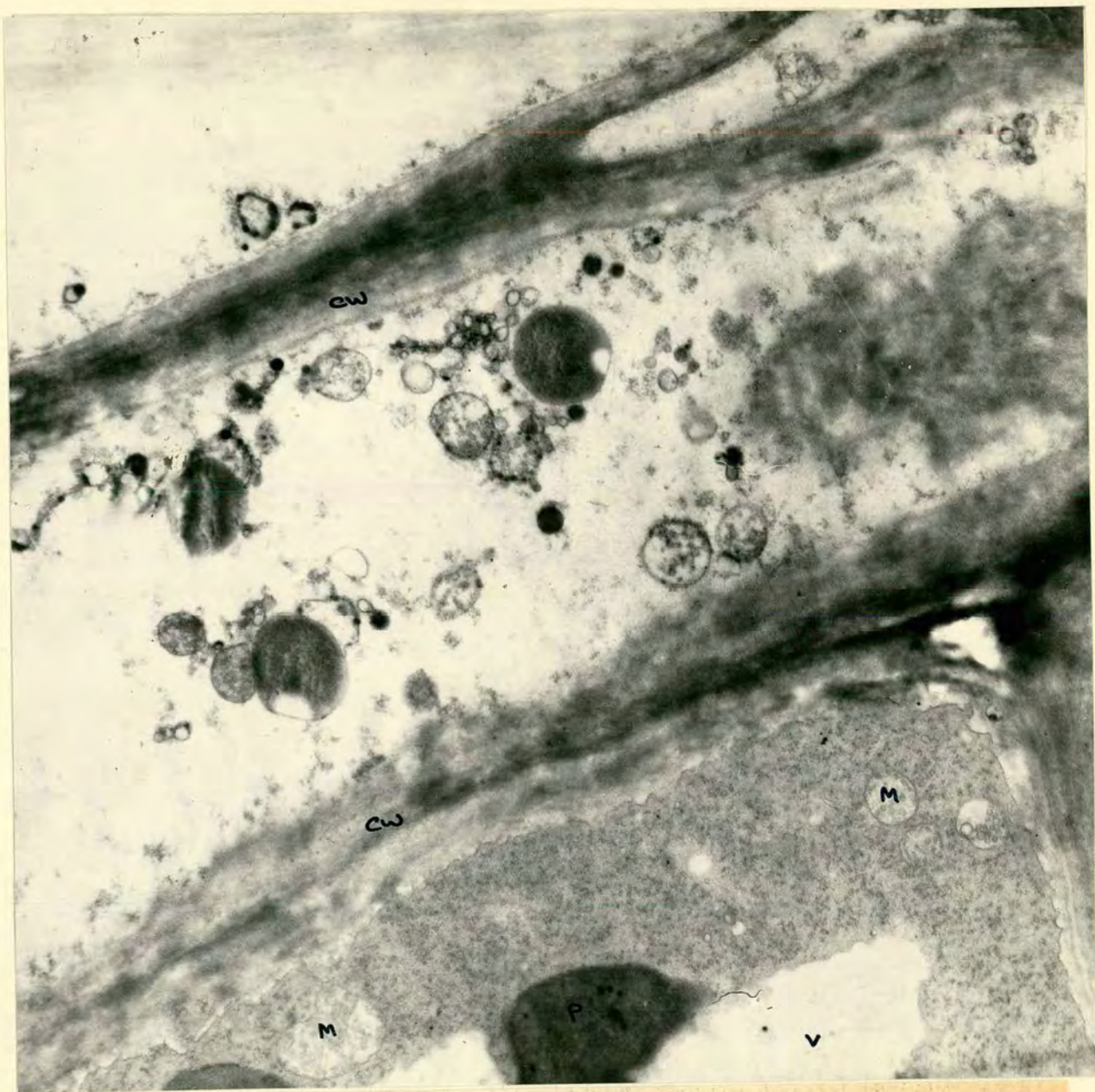
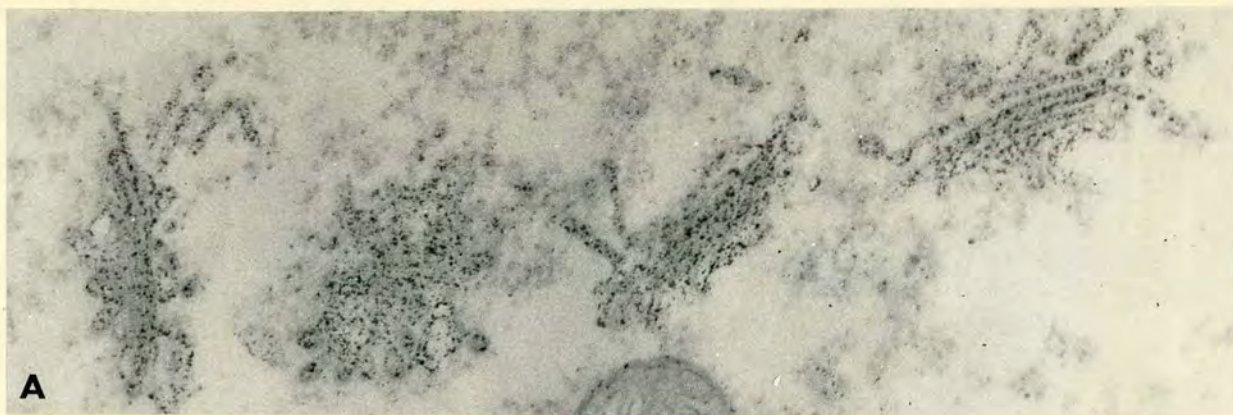
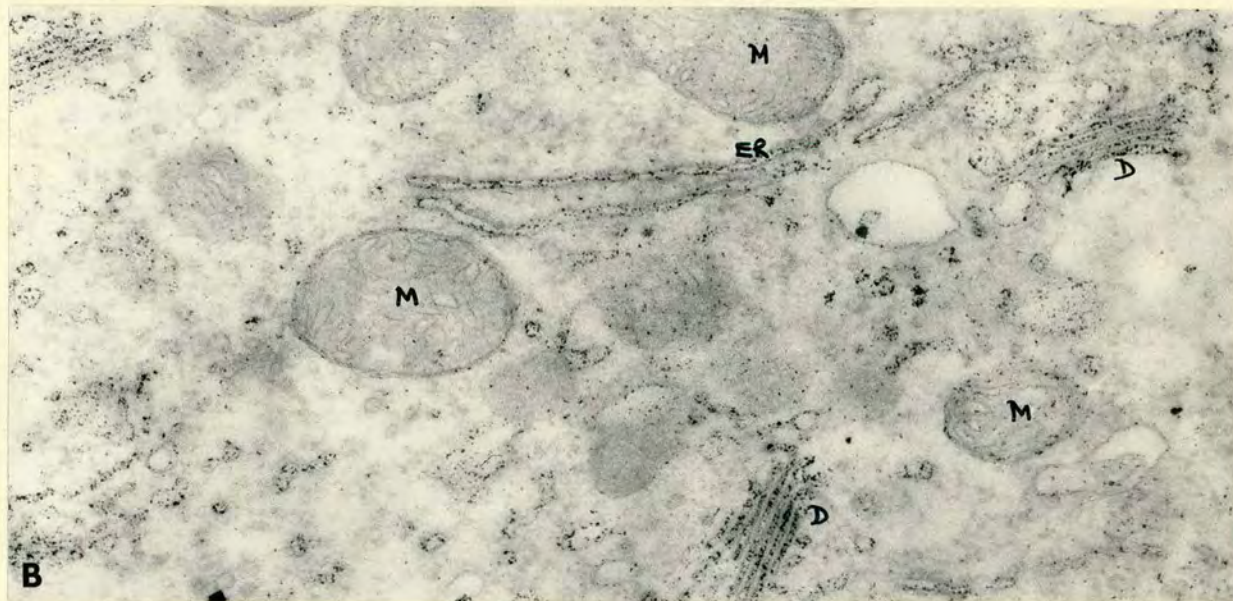


PLATE 27.

Tissue removed from the surface of a tuber explant cultured for 24 hours (Standard medium: Agar: 1). The cells presumably cut and damaged during excision of the explant contain a variety of vesicles, membranes and other electron dense material whereas the underlying cell contains apparently normal cell components. (G,Os,Pb) X 13,000.



A



B



C

PLATE 28.

Tissue cultured for 4 days (standard medium:Liquid:3)

Fixed in glutaraldehyde, incubated in a Gomori acid phosphate medium,
post fixed in osmium tetroxide. Sections examined unstained.

A Positive reaction in mitochondria. X 45,000.

Young cytoplasm. X 40,000.

B Positive reaction in mitochondria, vacuoles and ER. X 35,000.

C Positive reaction in mitochondria, vacuoles and ER. X 35,000.

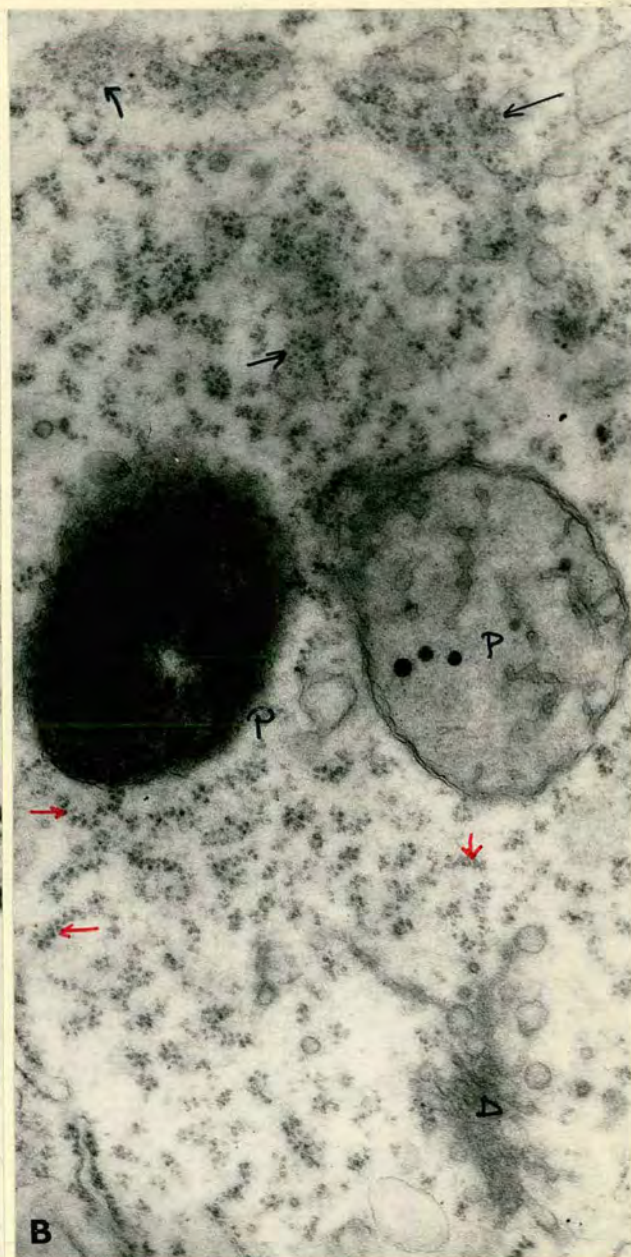
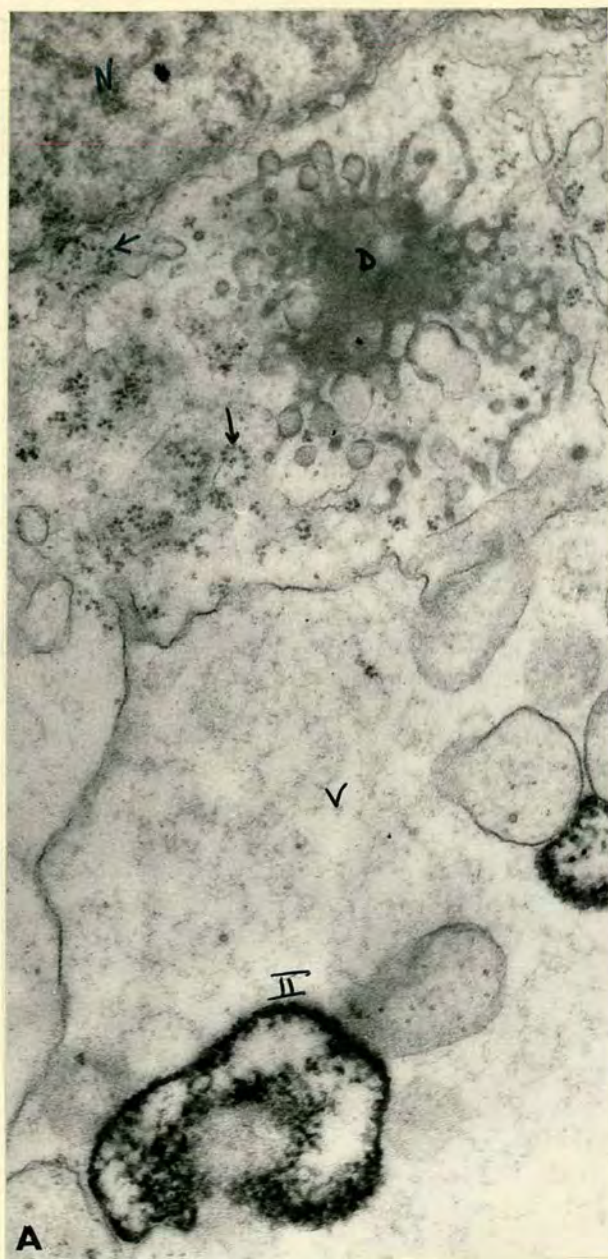


PLATE 29.

Tissue cultured for 24 hours (standard medium: Agar: 1) showing spiral and helical polysomes. The spiral ones (black arrows) are apparently bound to ER but the helical ones (red arrows) are free in the ground substance. (G, Os. Pb)

- A Vesicles of various sizes are connected to the dictyosomes. A fibrillar dense body (II) is shown in the vacuole. X 38,000.
 B Note the contrast in density of the two plastids. X 35,000.



PLATE 30.

Tissue cultured for 6 hours (standard medium: Agar :1) showing parts of several cells containing homogenous bodies(I) resembling lipid on the surface of the peripheral cytoplasm and in the vacuoles. These cells also have a very dense ground substance. (G,Os.Pb) X 11,000.

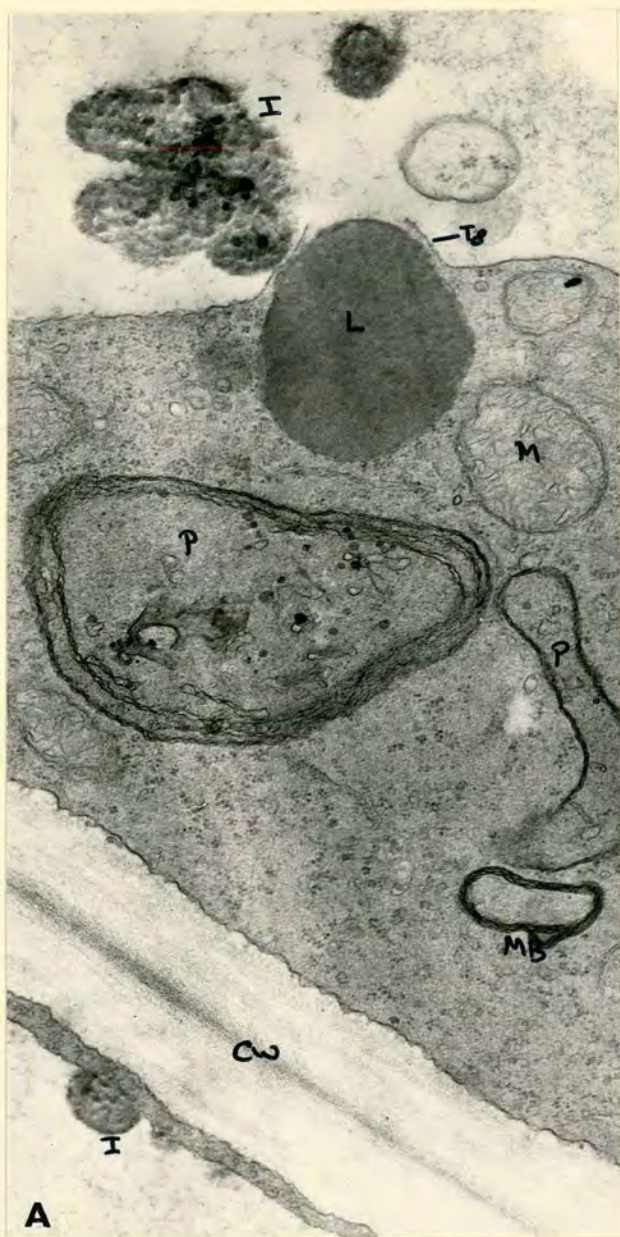


PLATE 31.

Tissue cultured 6 hours (standard medium: Agar: 1). The ground substance is fairly dense and contains whorls of membranes (myelin-like bodies). (G, Os. Pb)

A Homogenous bodies are present in the vacuole and the tonoplast is missing above a lipid body at the surface of the cytoplasm. The arrows indicate polysomes. X 28,000.

B Fibrillar material is present in the vacuole. One of the plastids a starch granule. X 32,000.

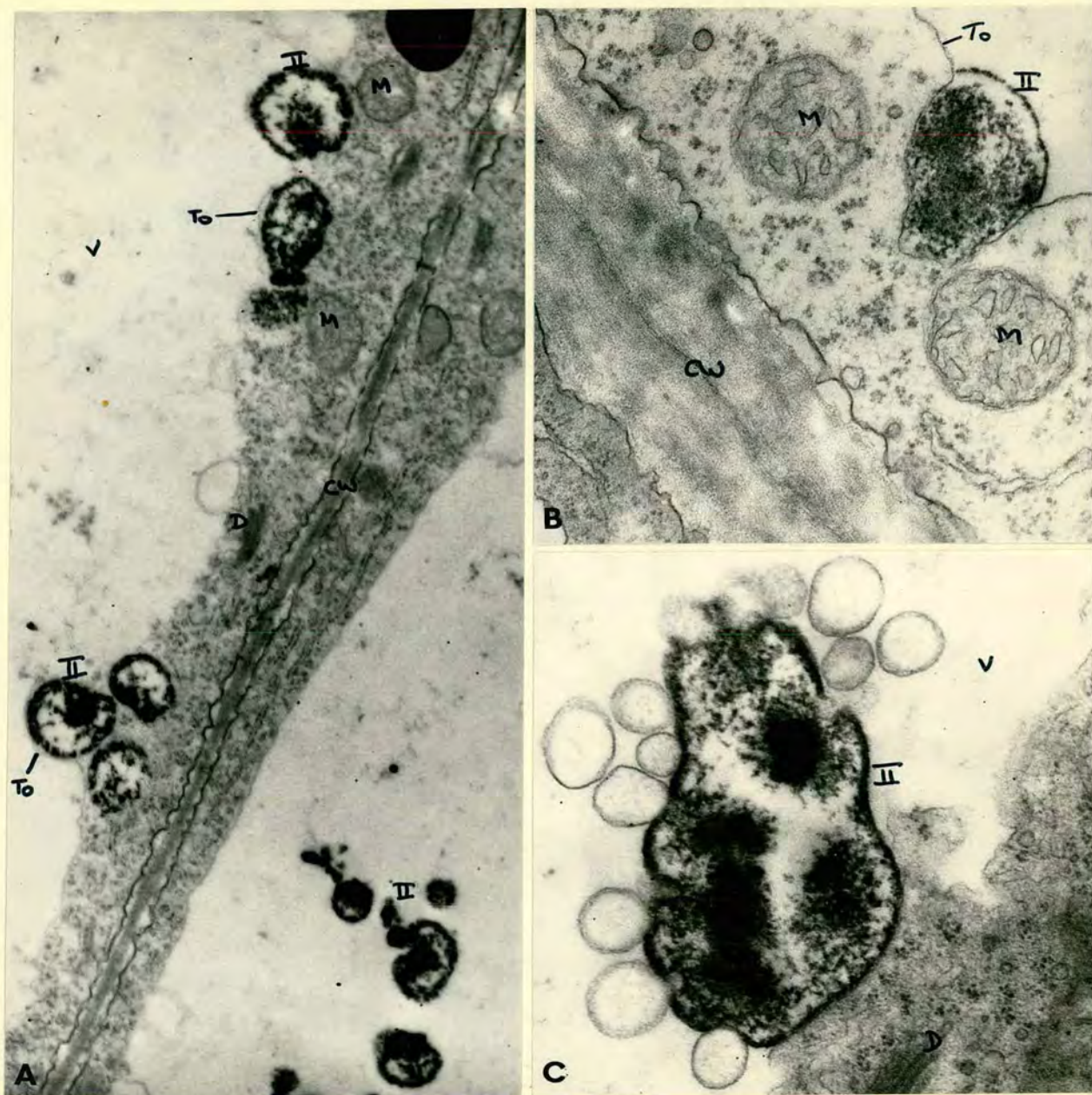


PLATE 32.

Cultured tissue (standard medium:Agar : 1) showing electron dense fibrillar bodies (II).

A Cultured 46 hours. The dense bodies are present both in the cytoplasm and in the vacuole. (G, Os.U,Pb) X 14,000.

B Cultured 24 hours. Note the variation in the density of the ground substance in the 2 cells. The dense body lies in the vacuole next to the tonoplast. (G, Os.Pb) X 40,000.

C Cultured 24 hours. The dense body is associated with vesicles (G, Os.Pb) X 39,000.

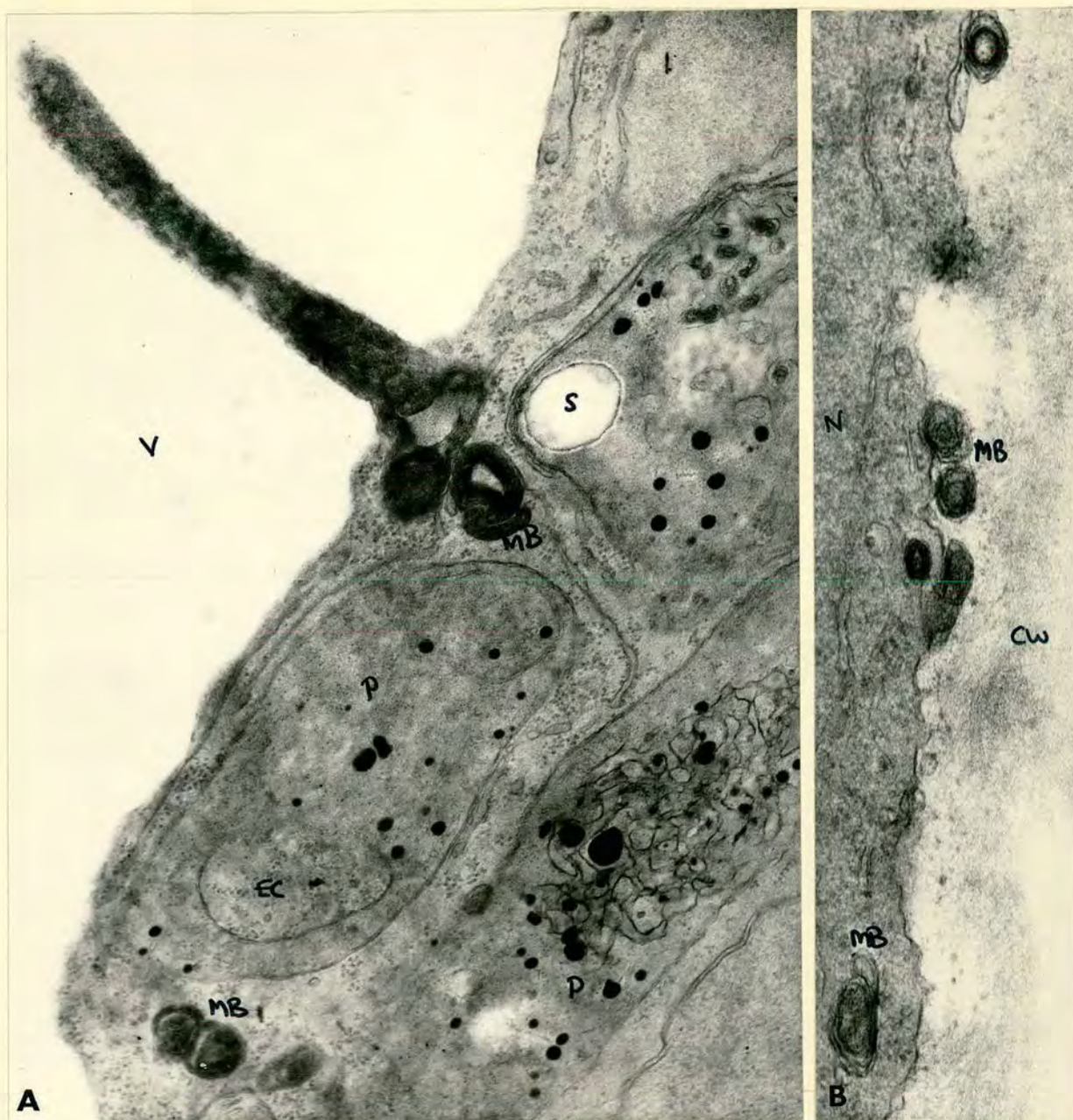


PLATE 33.

Cultured tissue (standard medium: Agar: 1) showing whorls of membranes (myelin-like bodies). The cells have a fairly electron dense ground substance. (G,Os.Pb.)

A Cultured 18 hours. Membrane whorls in the cytoplasm. The plastids contain starch. X 37,000.

B Cultured 24 hours. Membrane whorls in the cytoplasm and between the plasmalemma and cell wall. X 42,000.

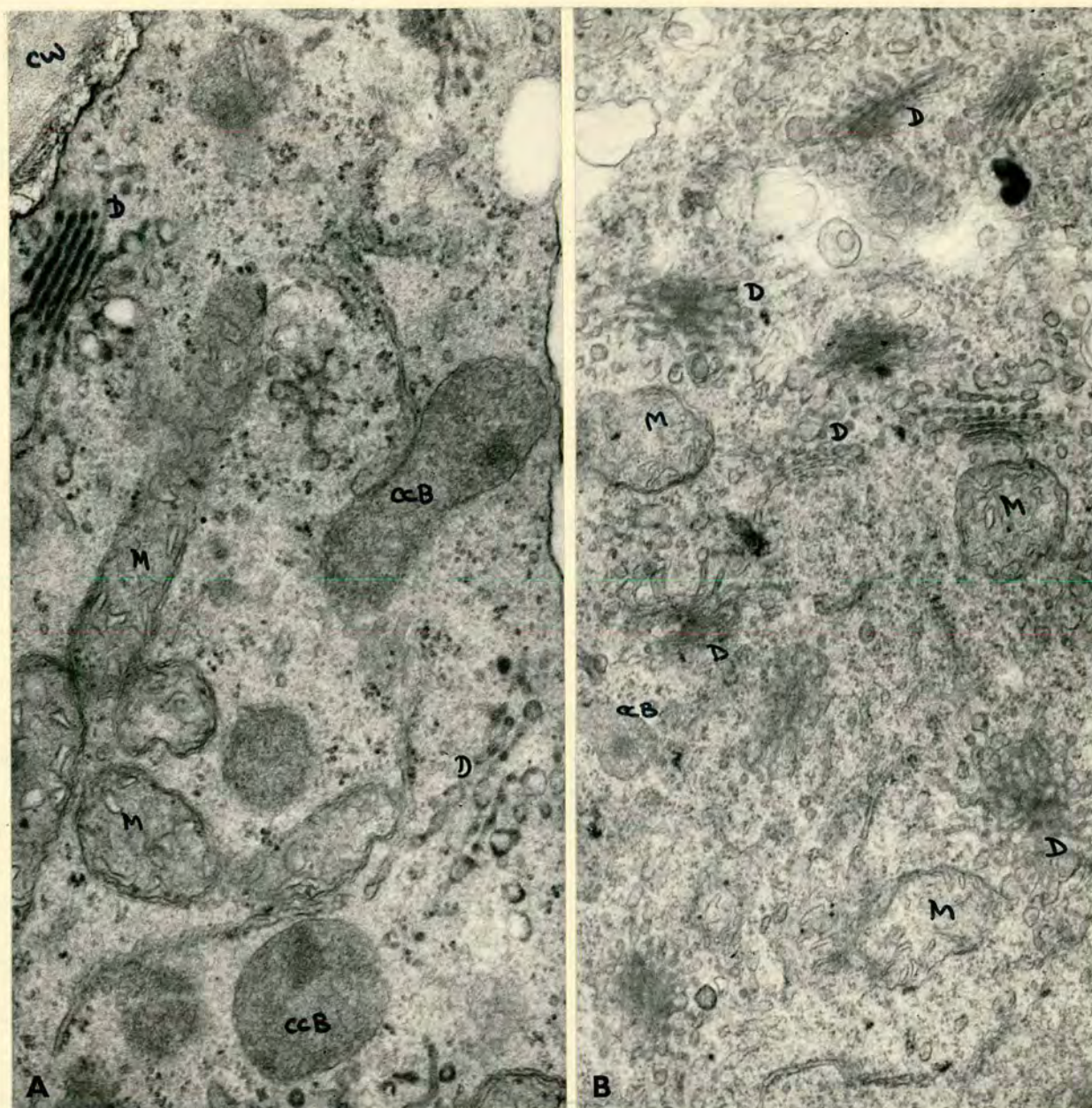


PLATE 34.

Cultured tissue showing sections of flat dictyosomes, simple mitochondria and small CCBs. (G, Os.U,Pb)

A Cultured 12 hours (standard medium: Agar: 1). The CCBs contain small crystals (arrowed) and a larger proportion of matrix.
X 43,000.

B Cultured 39 hours (No CM; 10^{-5} M 2.4.D : liquid :4). The group of dictyosomes are associated with many vesicles X 28,000.

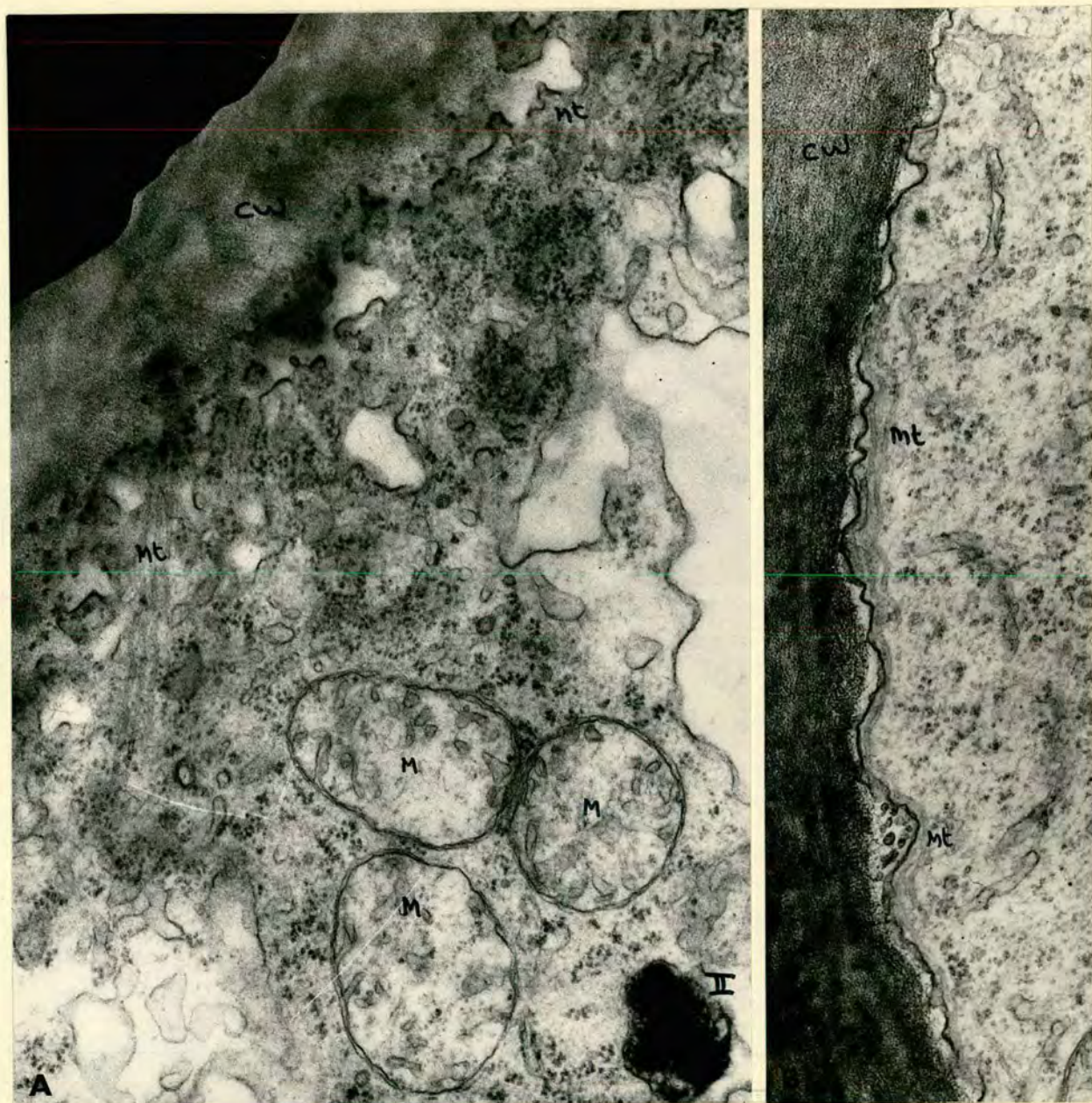


PLATE 35.

Cultured tissue showing microtubules near the cell wall.

A Cultured 24 hours (standard medium: Agar: 1). The mitochondria contain few cristae in a transparent matrix. Polysomes are abundant. (G,Os,Pb). X 39,000.

B Cultured 39 hours. (No CM; 10^{-5} M 2.4.D: Liquid: 4) (G,Os,U,Pb) X 43,000.

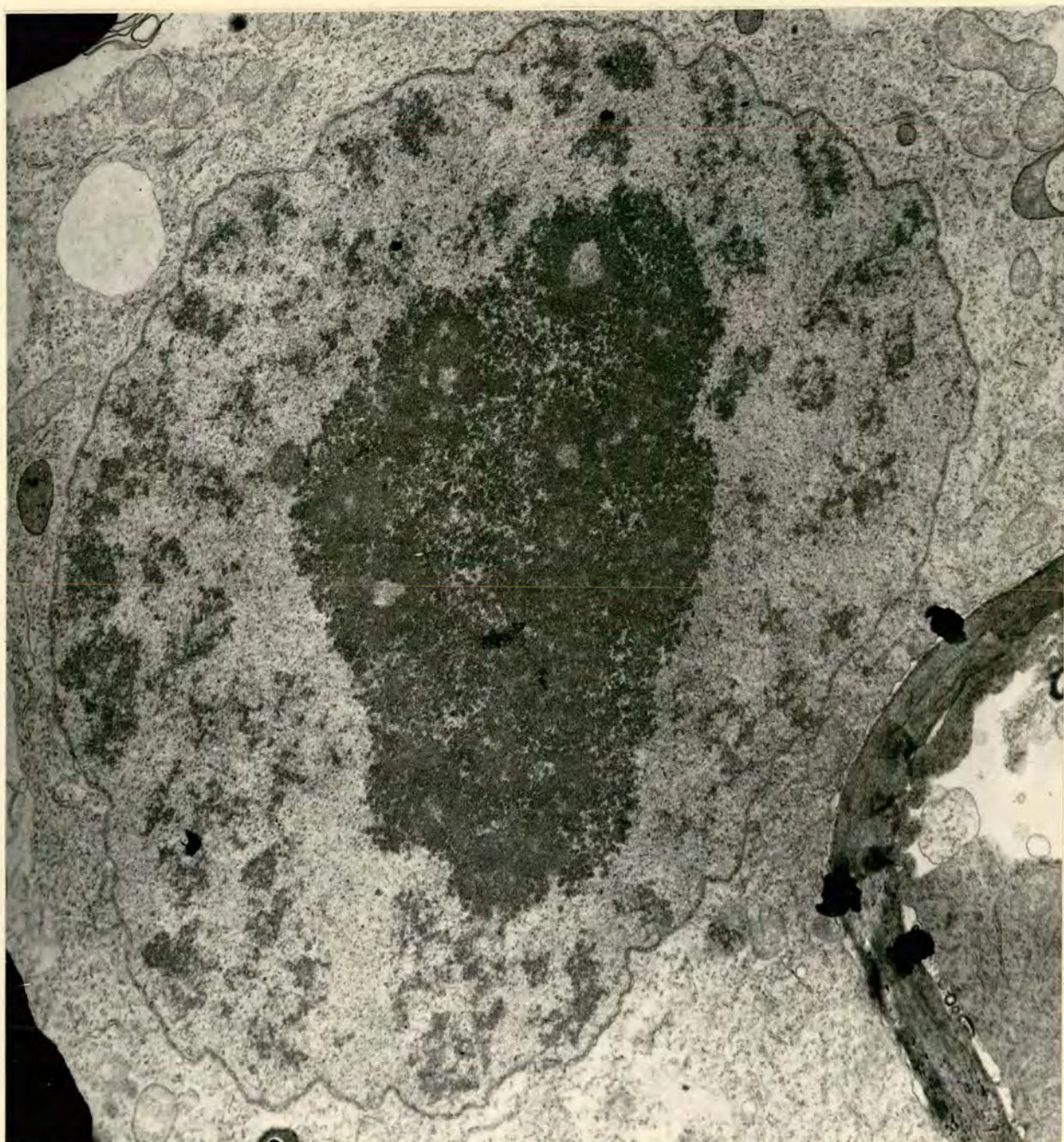


PLATE 36.

Tissue cultured 39 hours (No CM; 10^{-5} 2.4D :Liquid:4). The rounded peripheral nucleus contains aggregates of chromatin and a large loosely packed nucleolus. (G, Os. U,Pb) X 11,000.

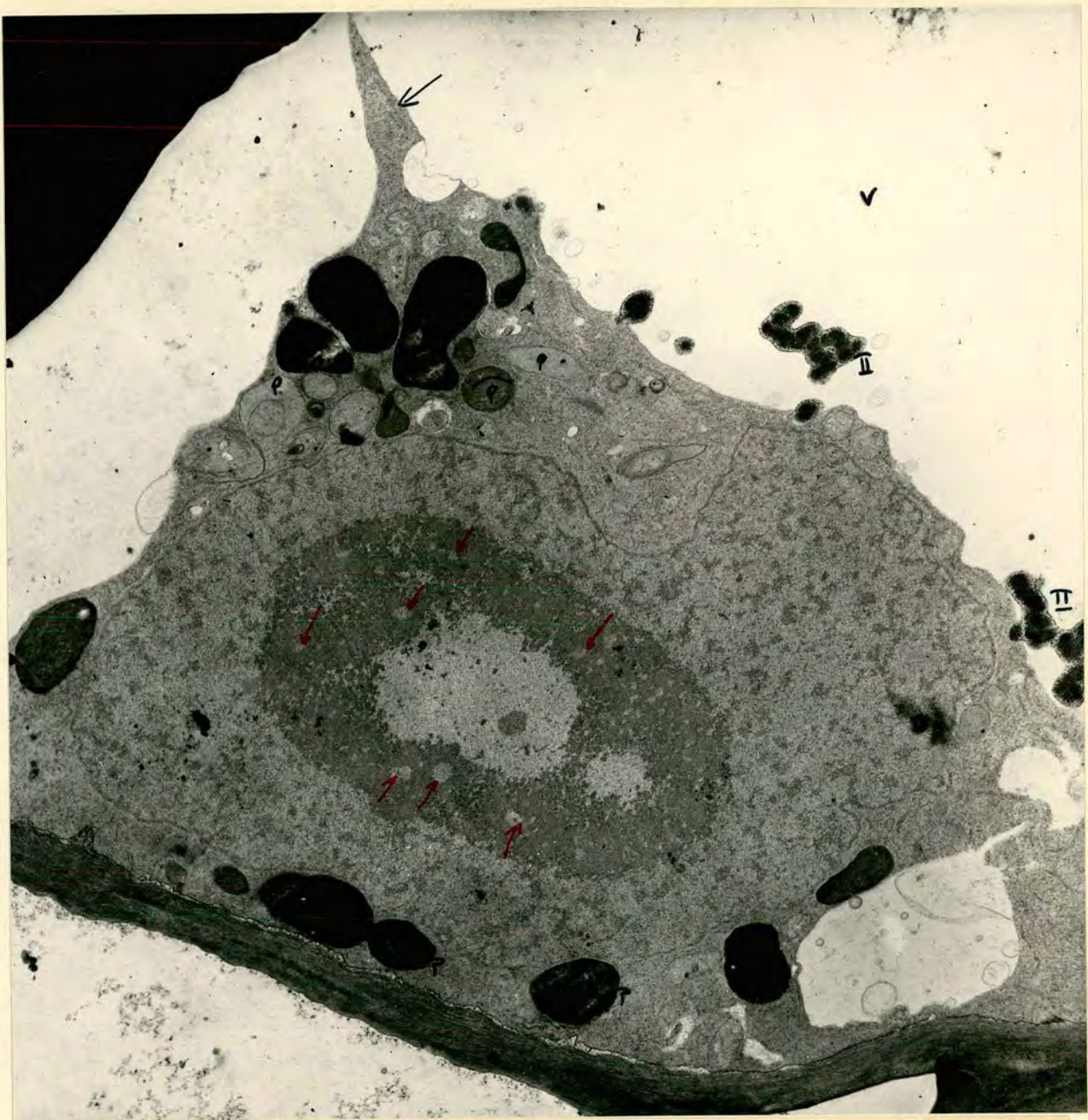


PLATE 37.

Tissue cultured 39 hours (No CM; 10^{-5} M 2,4D; Liquid :4). The nucleolar material is loosely packed. The nucleolus contains a large central electron transparent region surrounded by granular material. ^{smaller} Similar transparent regions (red arrows) are surrounded by fibrillar material. Part of a cytoplasmic strand is marked by the black arrow. Note the variation in density of the plastids. (G,Os.U,Pb) X 10,000.



PLATE 38.

Tissue cultured 39 hours (No CM; 10^{-5} M2.4D : Liquid: 4)

Early prophase. The nucleus is lying in the vacuole on a stout column of cytoplasm (See Plate 39). (G,Os,U,Pb) X 5,000.

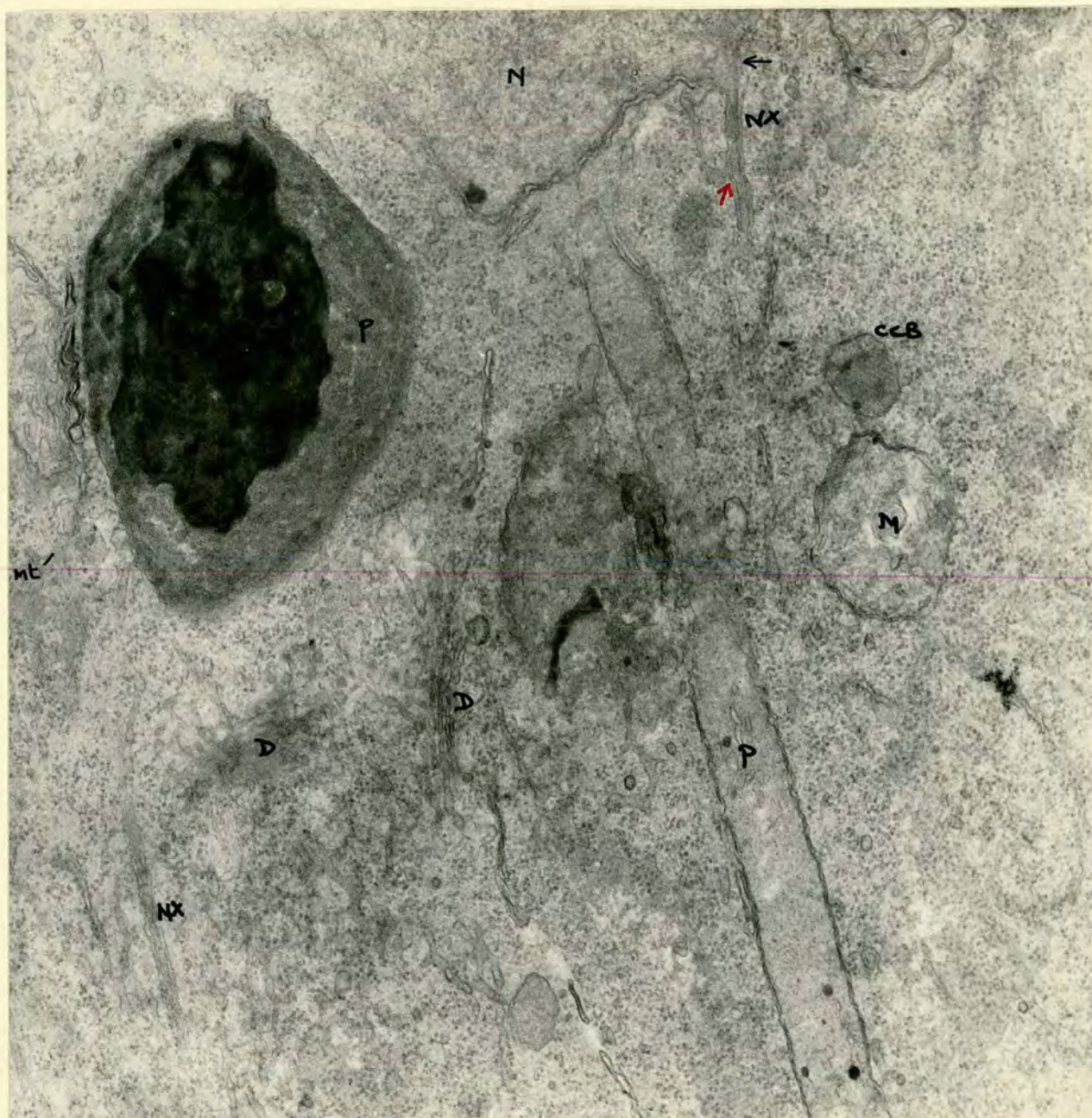


PLATE 39.

Cultured 39 hours. Early prophase.

Enlargement of Plate 38 showing part of the cytoplasm lying between the nucleus and the cell wall. A possible connection between a nuclear extension and the nucleus is shown (black arrow) and a possible connection between the extension and ER (red arrow).

(G, Os, U, Pb) X 33,000.

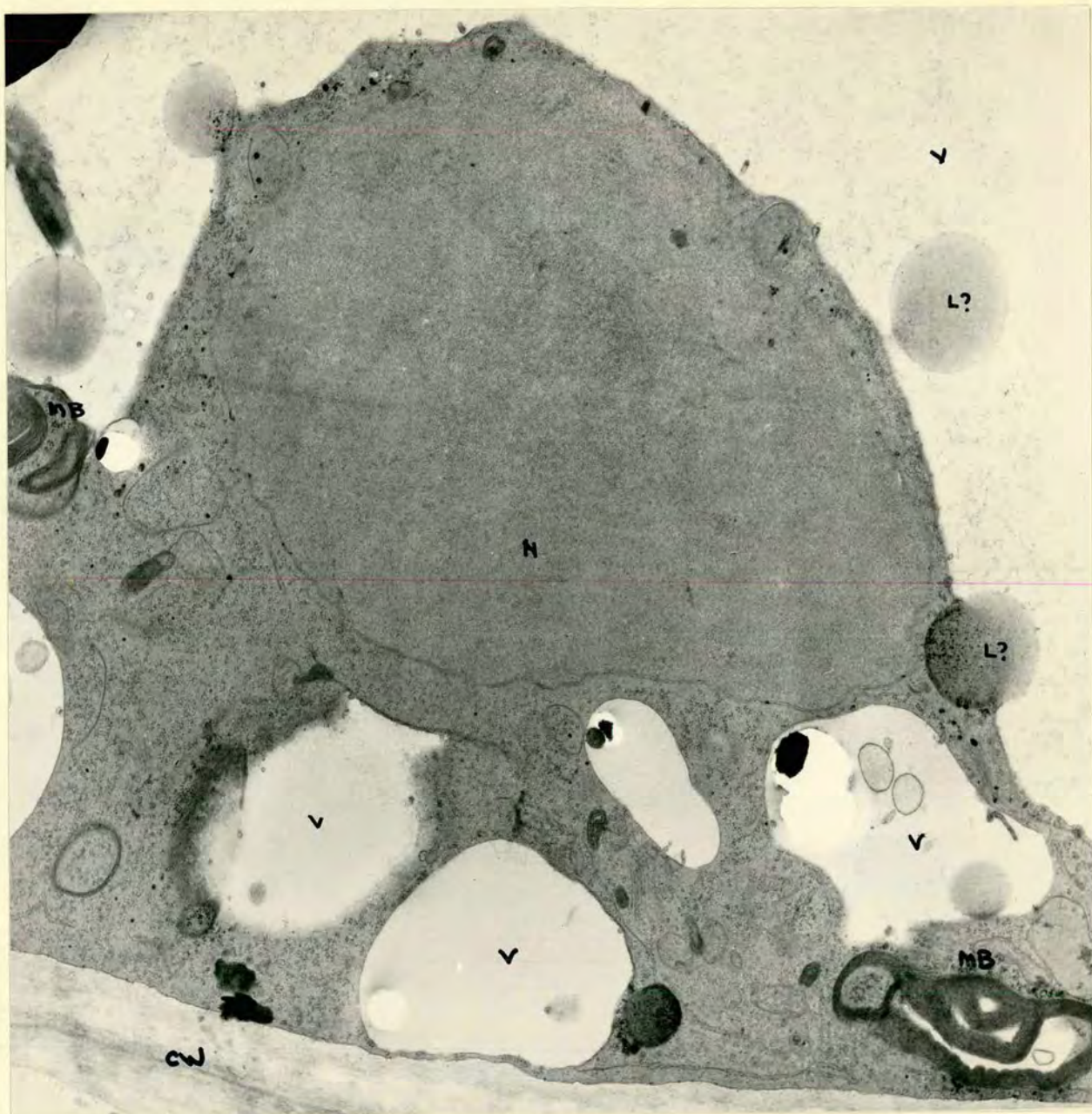


PLATE 40.

Tissue cultured 24 hours (standard medium:Agar:1). Early prophase. The nucleus is held away from the cell wall by several cytoplasmic strands. The cytoplasm contains whorls of membranes (myelin-like bodies). (G,Os.Pb) X 18,000.

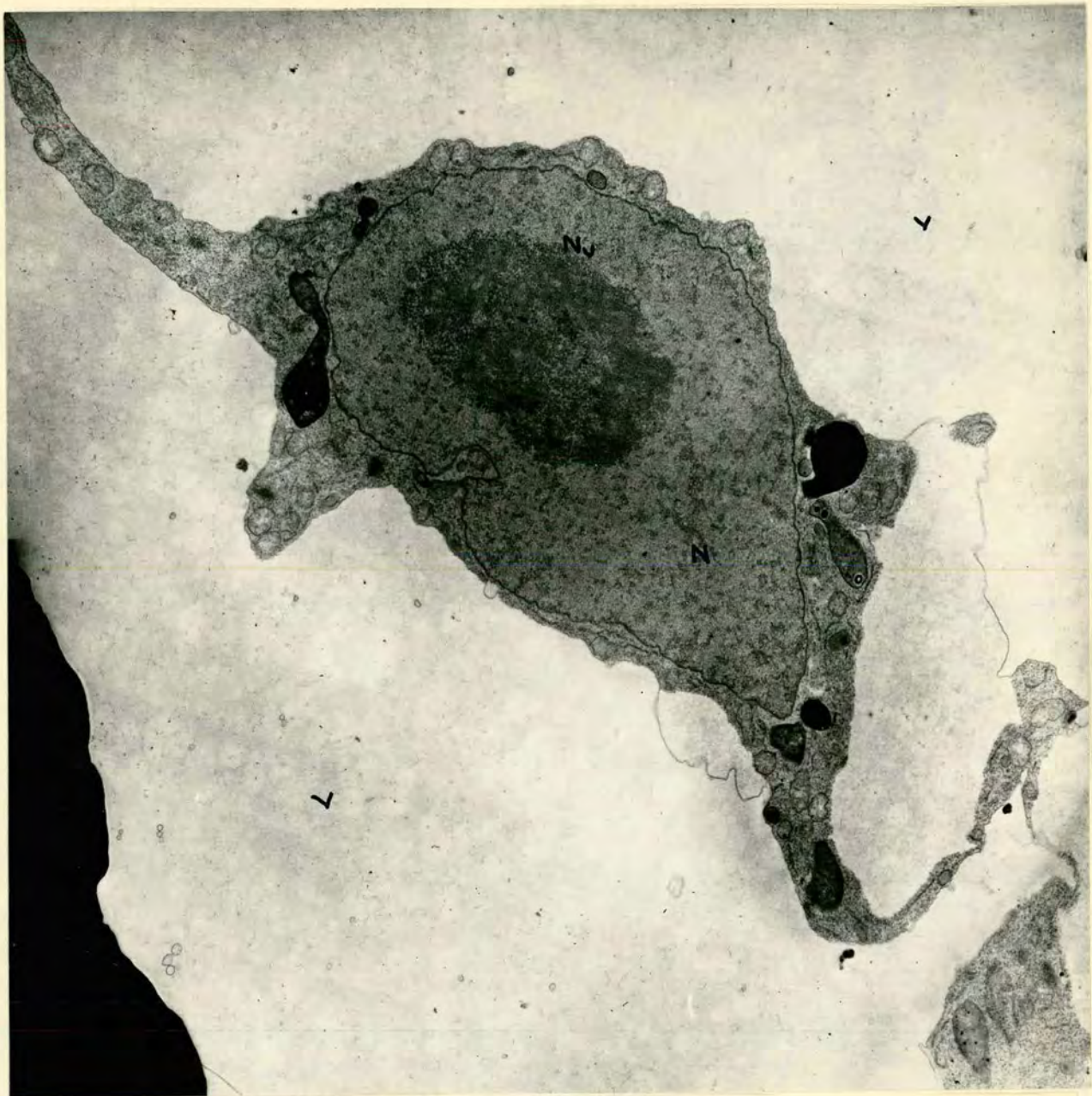


PLATE 41.

Tissue cultured 39 hours (No CM; 10^{-5} M 2.4D; Liquid ;4). Early prophase. The rounded nucleus is suspended in the vacuole by a narrow transvacuolar cytoplasmic strand (See Plate 42). The nucleolar material is loosely packed. (G,Os.Pb) X 5,000.



PLATE 42.

Cultured 39 hours. Early prophase.

Enlargement of Plate 41 showing part of the cytoplasmic strand containing nuclear extensions. The red arrow indicates a possible connection between a nuclear extension and ER. (G,Os.Pb) X 39,000.

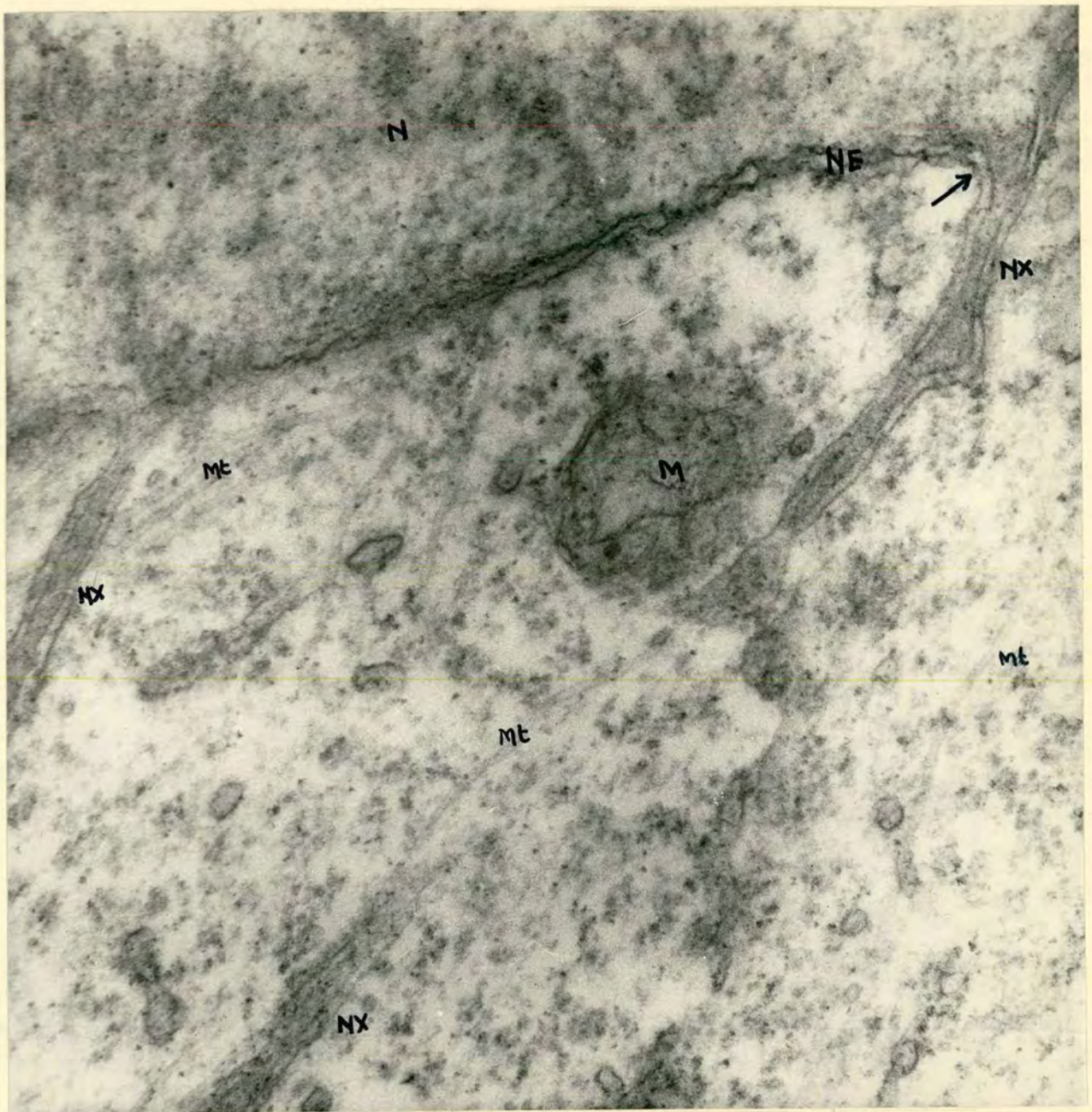


PLATE 43.

Tissue cultured 7 days in the light (No CM: Agar; 1) showing nuclear extensions and microtubules. One nuclear extension (arrowed) communicates with the nucleus. (G,Os.Pb) X 75,000.

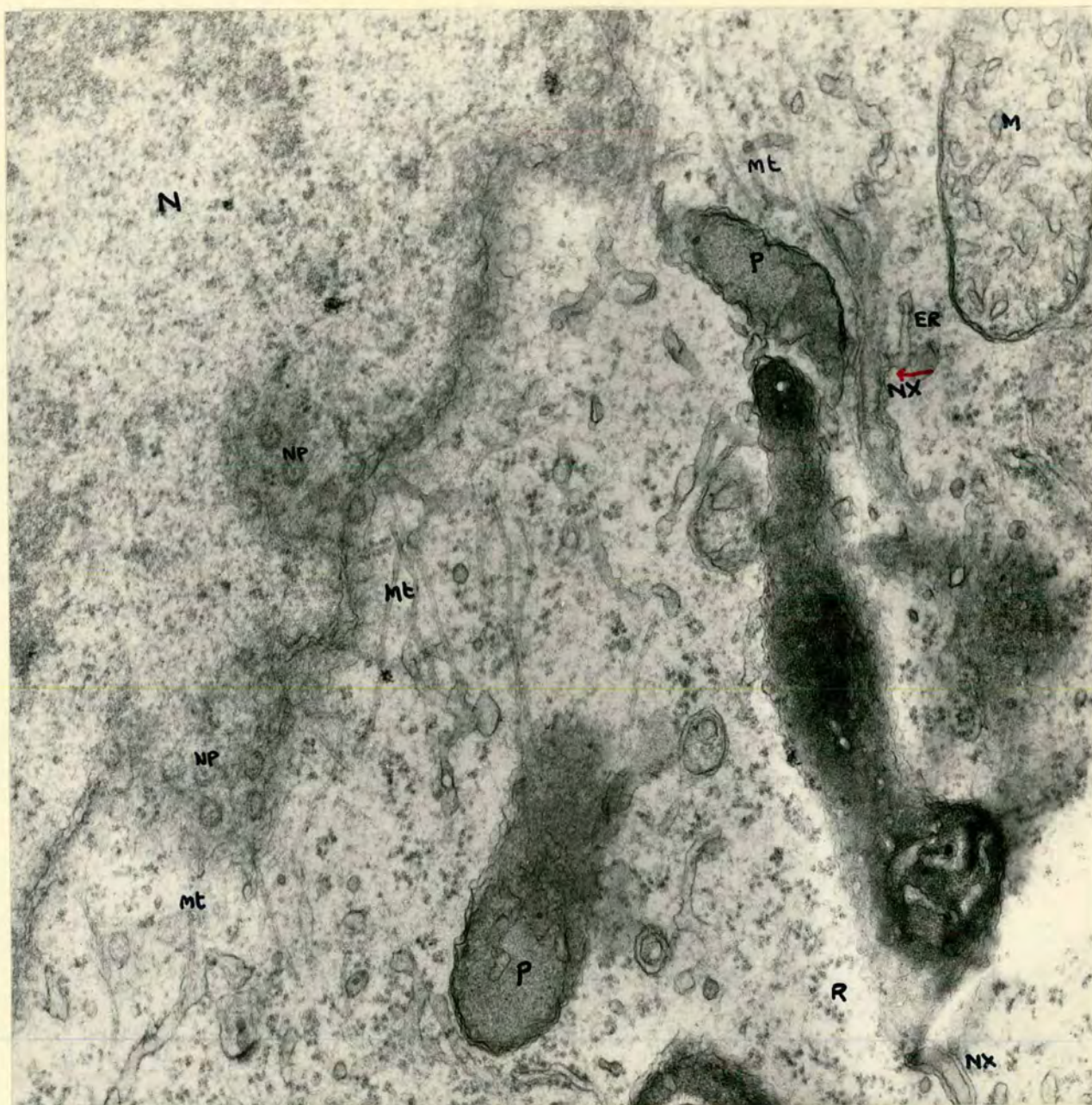


PLATE 44.

Tissue cultured 39 hours (No CM; 10^{-5} M 2.4.D :Liquid: 4)

Prophase: Showing microtubules surrounding part of the nucleus. Nuclear extensions are shown and a possible connection with ER is indicated by the red arrow. (G,Os.U,Pb) X 43,000.

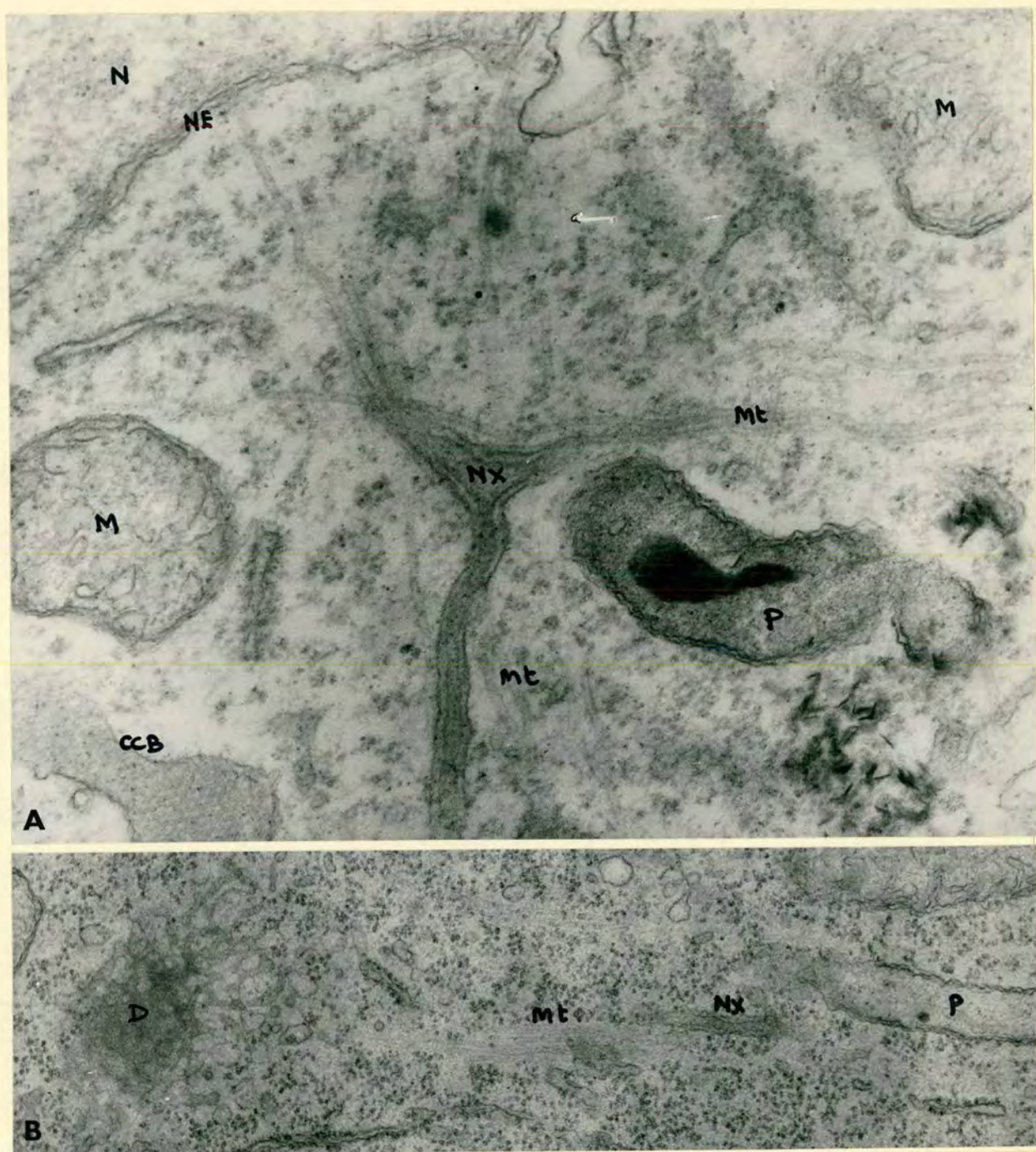


PLATE 45.

- Nuclear extensions and microtubules in cultured tissue. (G, Os. U, Pb)
- A Tissue cultured 7 days, (No CM; Agar: 1) in the light, showing a branching nuclear extension associated with microtubules. X 72,000.
- B Tissue cultured 39 hours (No CM; 10^{-5} M 2.4.D: Liquid: 4). Microtubules and part of a nuclear extension are shown lying parallel to the direction of the cytoplasmic strand. X 42,000.



PLATE 46.

Tissue cultured 39 hours (No CM; 10^{-5} M 2,4D; Liquid: 4).

Metaphase or Anaphase. Cytoplasmic strands are suspending the nuclear material. Mitochondria, plastids and dictyosomes surround the mitotic spindle region. (G,Os,U,Pb) X 8,000.

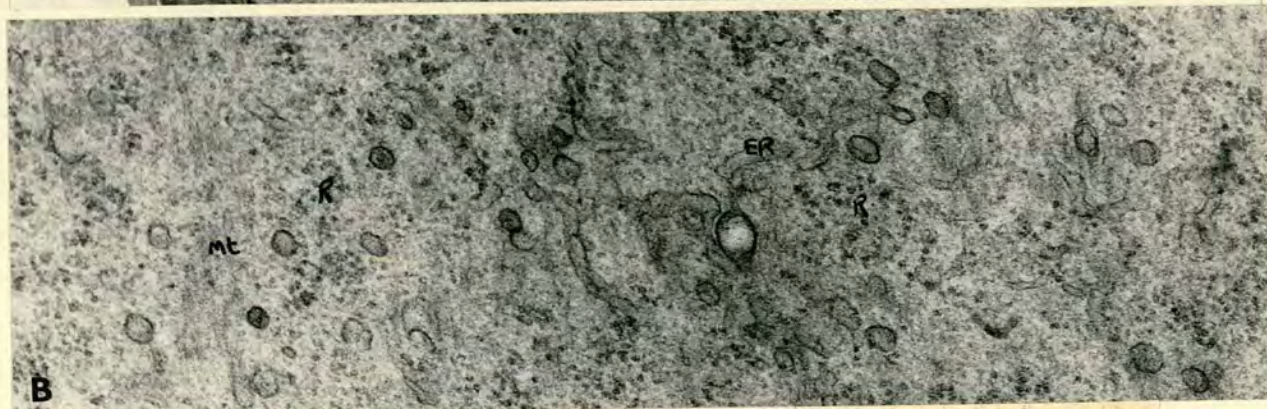
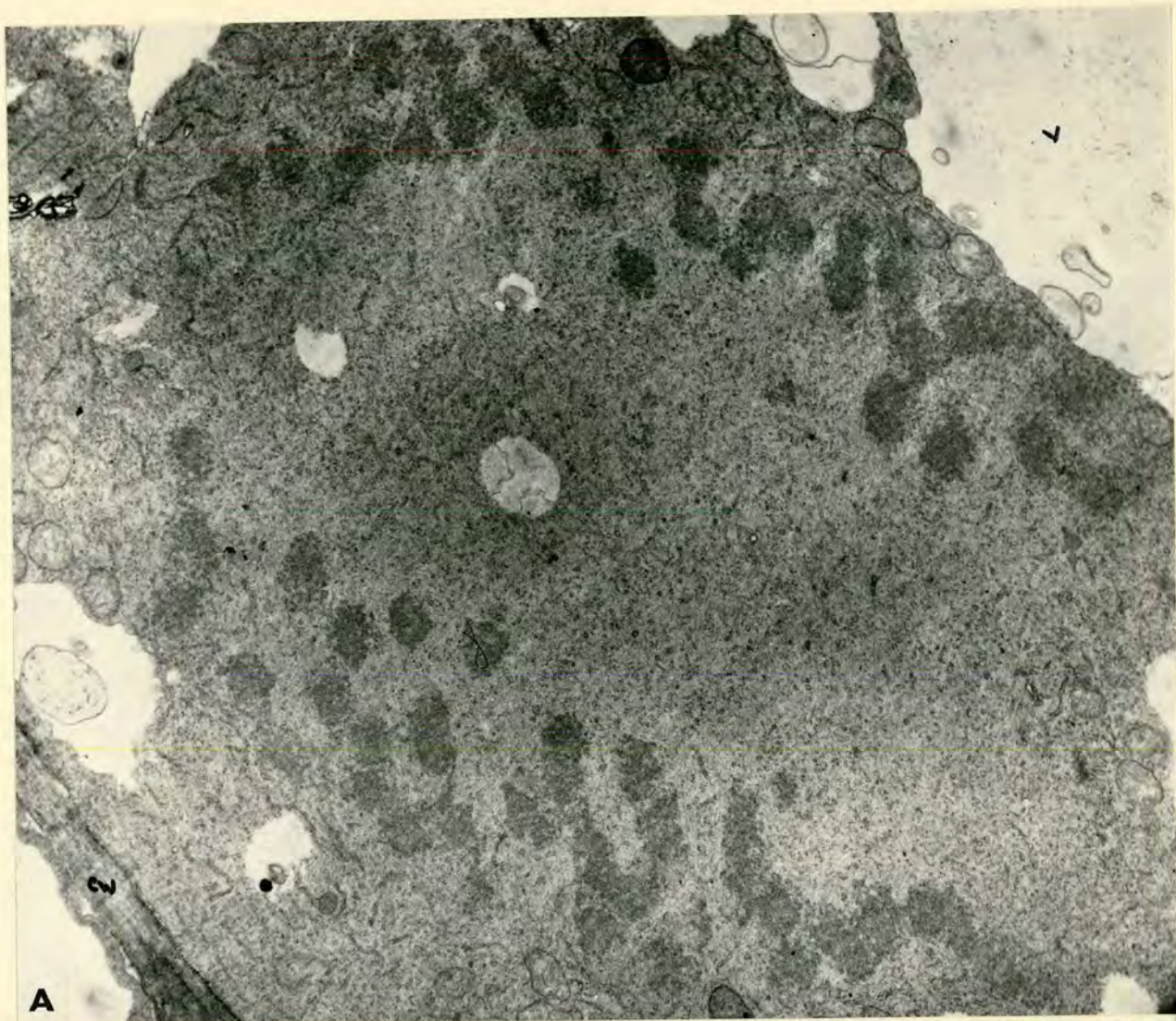


PLATE 47.

Tissue cultured 39 hours (No CM; 10^{-5} M 2.4D; Liquid: 4)

Anaphase. (G, Os, U, Pb)

A The chromatids have separated and moved to opposite broad poles.

X 8,000.

B Enlargement of the equatorial plate region. Vesicles, ER, microtubules and ribosomes are shown. X 43,000.



PLATE 48.

Tissue cultured 39 hours (No CM; 10^{-5} M 2.4.D: Liquid: 4)

Early Telophase. The newly formed nucleus lies near the developing cell plate (arrowed). The nuclear profile contains fibrous nucleoli, dispersed granules, membrane bound spaces and whorls of membranes (See Plate 49). (G,Os.U,Pb) X 11,000.



PLATE 49.

Tissue cultured 39 hours.

Telophase. Enlargement of Plate 48 showing a nucleolus (mostly composed of fibrils), scattered granules around fibrous chromatin (arrowed), membrane bound spaces and whorls of membranes (myelin-like bodies) (G,Os.U,Pb) X 36,000.

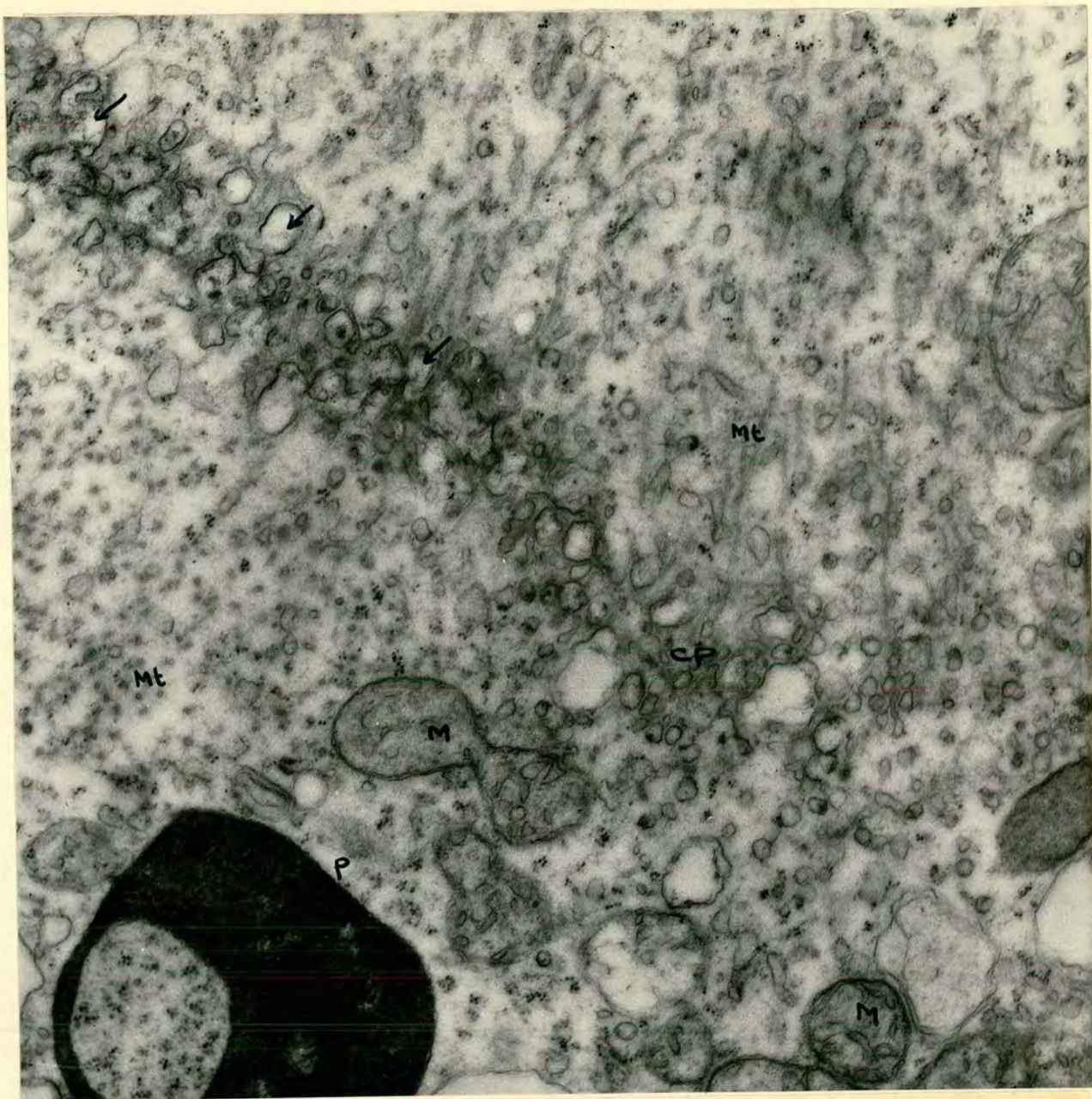


PLATE 50.

Tissue cultured 30 hours (Standard medium: Agar: 1) showing the growing end of a developing cell plate composed of vesicles of various sizes. Some of the vesicles (arrowed) contain fibrils. Microtubules are abundant. (G,Os.U,Pb) X 28,000.

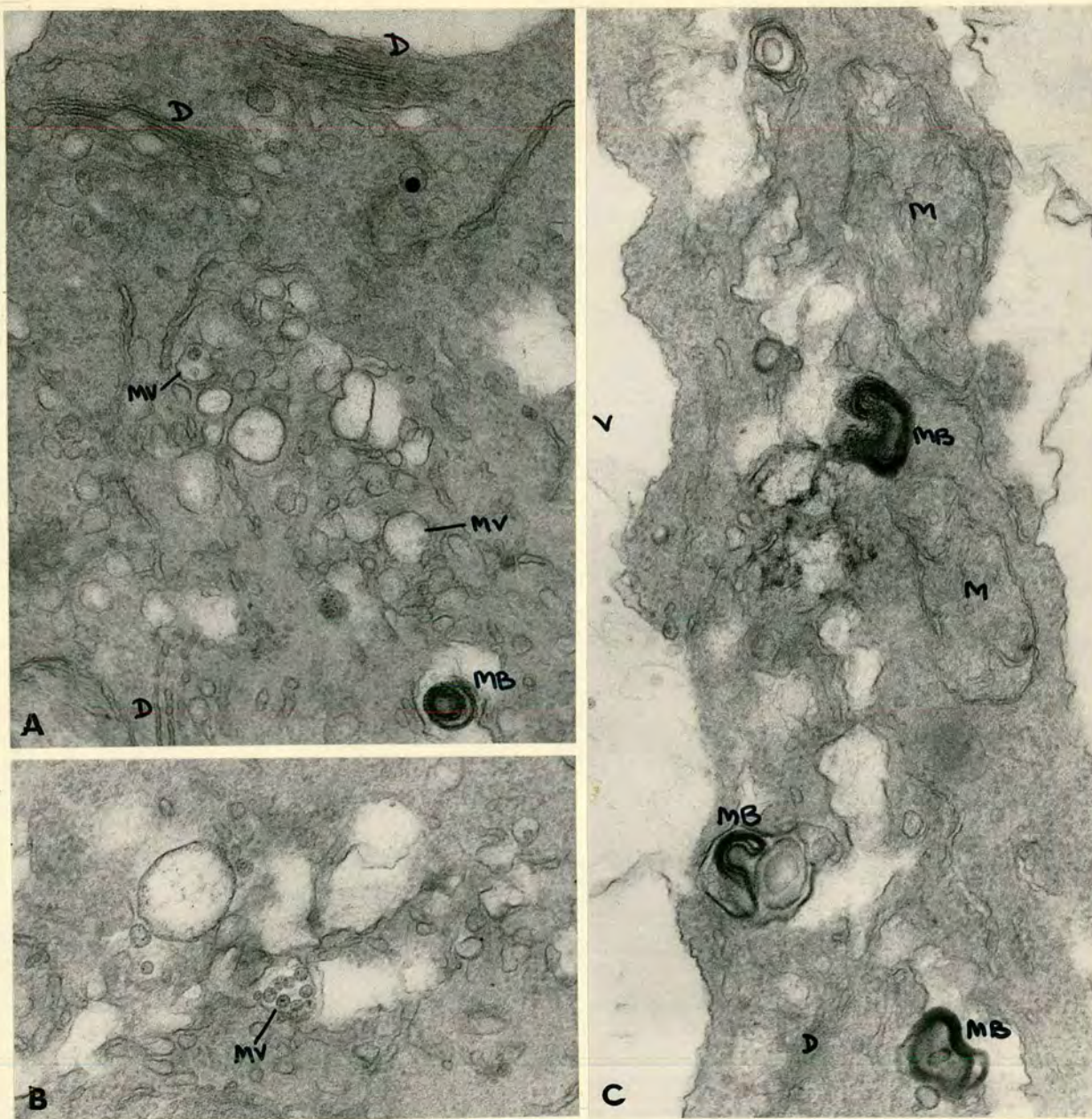


PLATE 51.

Cell plates in tissue cultured for 39 hours (No CM; 10^{-5} M 2.4.D; Liquid: 4) (G,Os.U,Pb) X 42,000.

A Growing end composed of vesicles of various sizes, including multivesicular bodies, and a myelin-like body. Dictyosomes surround the plate.

B A multivesicular body in the cell plate region.

C Whorls of membranes (myelin-like bodies) near the cell plate.

Large fused vesicles in the plate contain fine dense fibrils.



PLATE 52.

Tissue cultured 39 hours. (NoCM; 10^{-5} M 2.4.D; Liquid: 4).

After completion of first division. The nucleolar granules are now compacted around the fibrillar portions. The new cell wall is complete and contains dense fibrils. Paramural bodies are shown. (G,Os.Pb)
X 13,000.

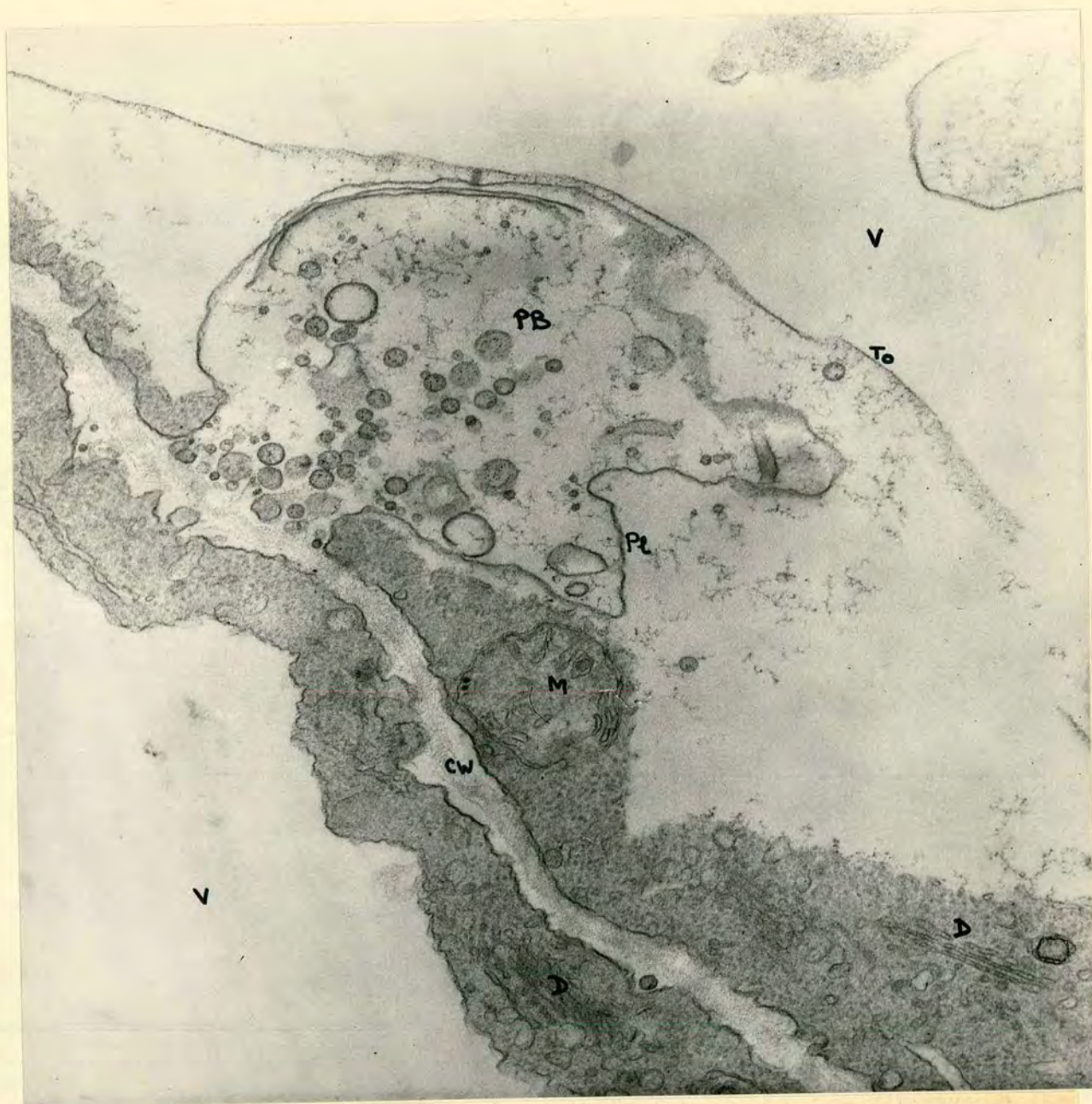


PLATE 53.

Tissue cultured 39 hours (No CM; 10^{-5} M 2,4-D; Liquid: 4) showing a paramural body, containing vesicles and fibrils, near newly formed cell wall. The tonoplast is detached in this cell and the paramural body apparently swollen. (G, Os. Pb) X 36,000.

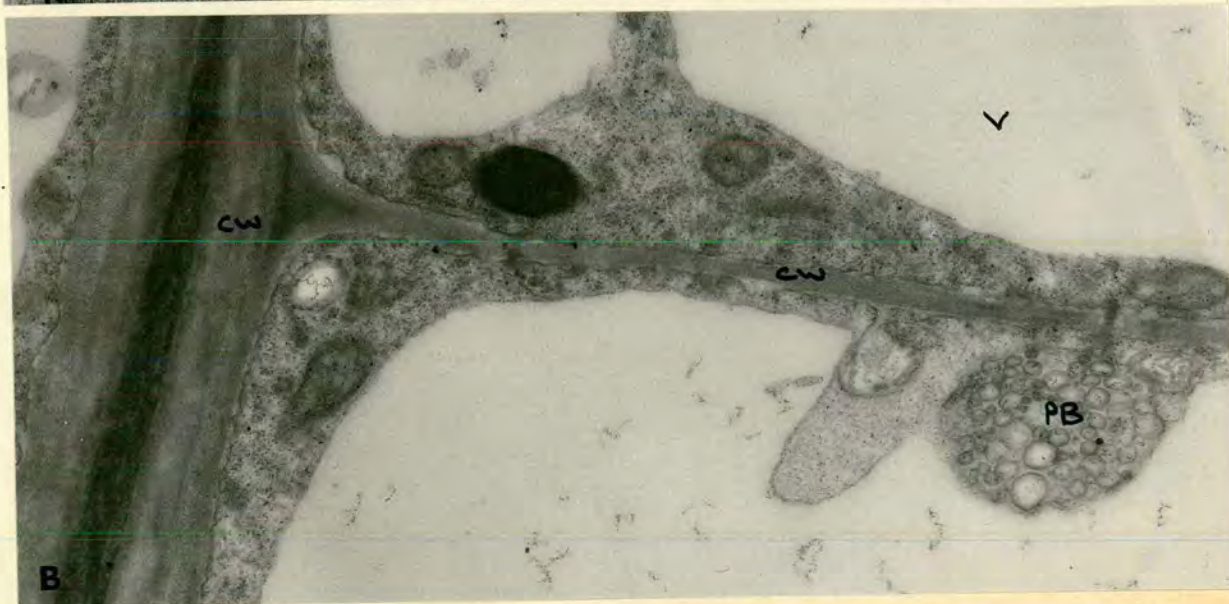
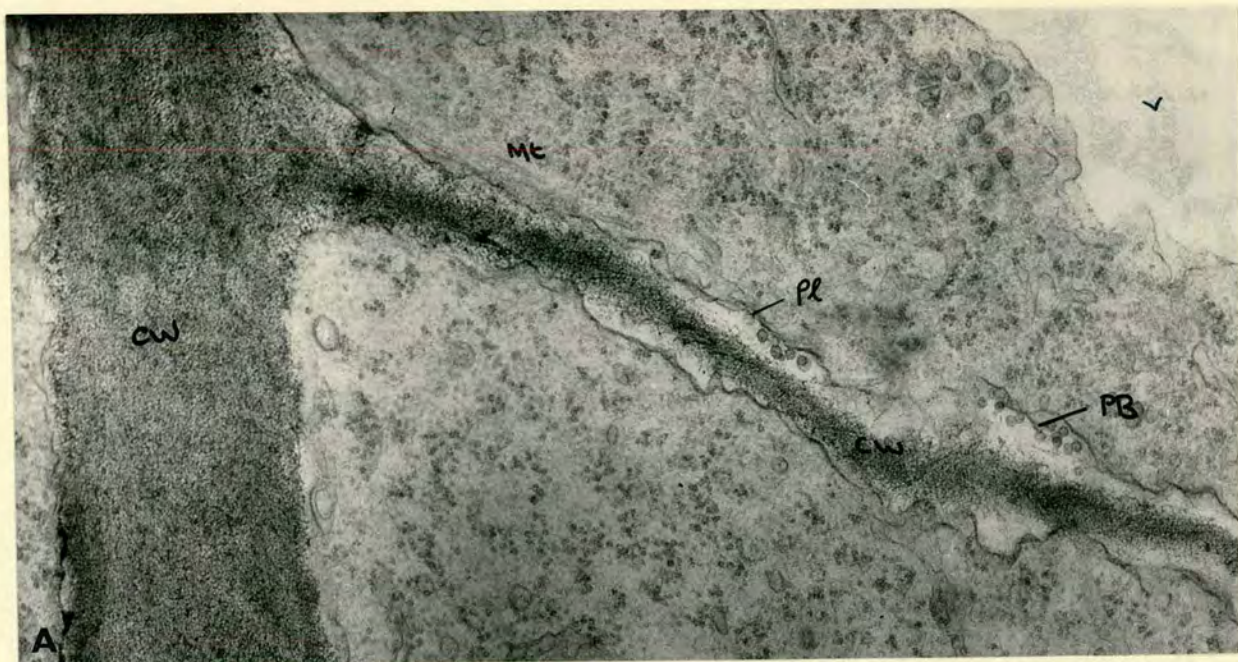


PLATE 54.

Cultured tissue showing connections between narrow newly formed cell walls and wider parent cell walls.

A Cultured 39 hours (No CM; 10^{-5} 2.4.D; Liquid: 4). Microtubules are shown in the cytoplasm near the plasmalemma and vesicles between the plasmalemma and cell wall. The fibrous nature of this wall can be seen (G,Os,U,Pb) X 43,000.

B Cultured 9 days (7 dark + 2 light) (No CM; Agar: 1). A structure (arrowed) similar to a paramural body appears to be situated in the cytoplasm (G,Os,Pb). X 20,000.

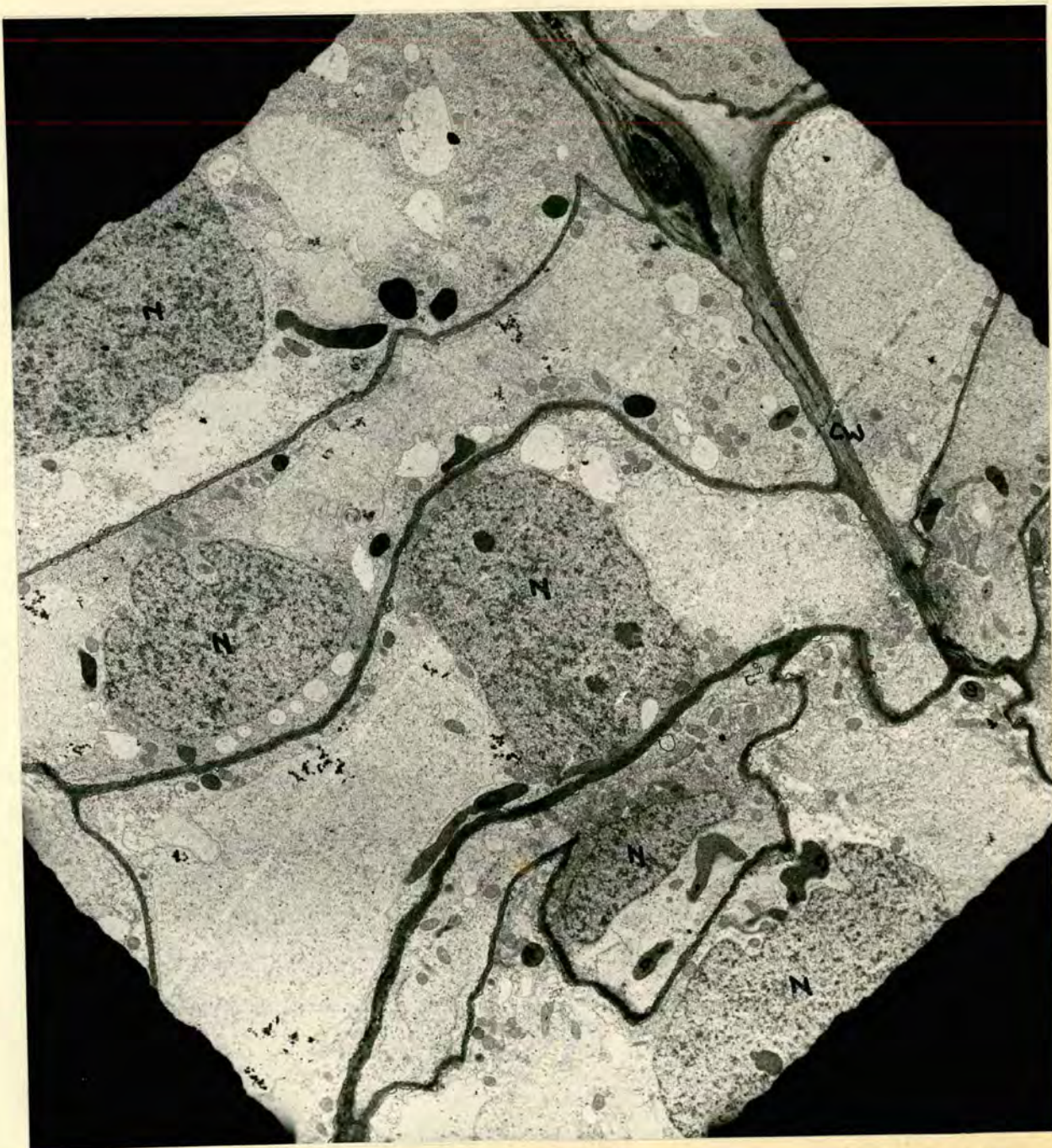


PLATE 55.

Tissue cultured 6 days (standard medium: Liquid: 2) showing part of a row of callus cells, resembling a cambium. The cells were at right angles to the surface of the explant. (G, Os. U, Pb) X 3,500.

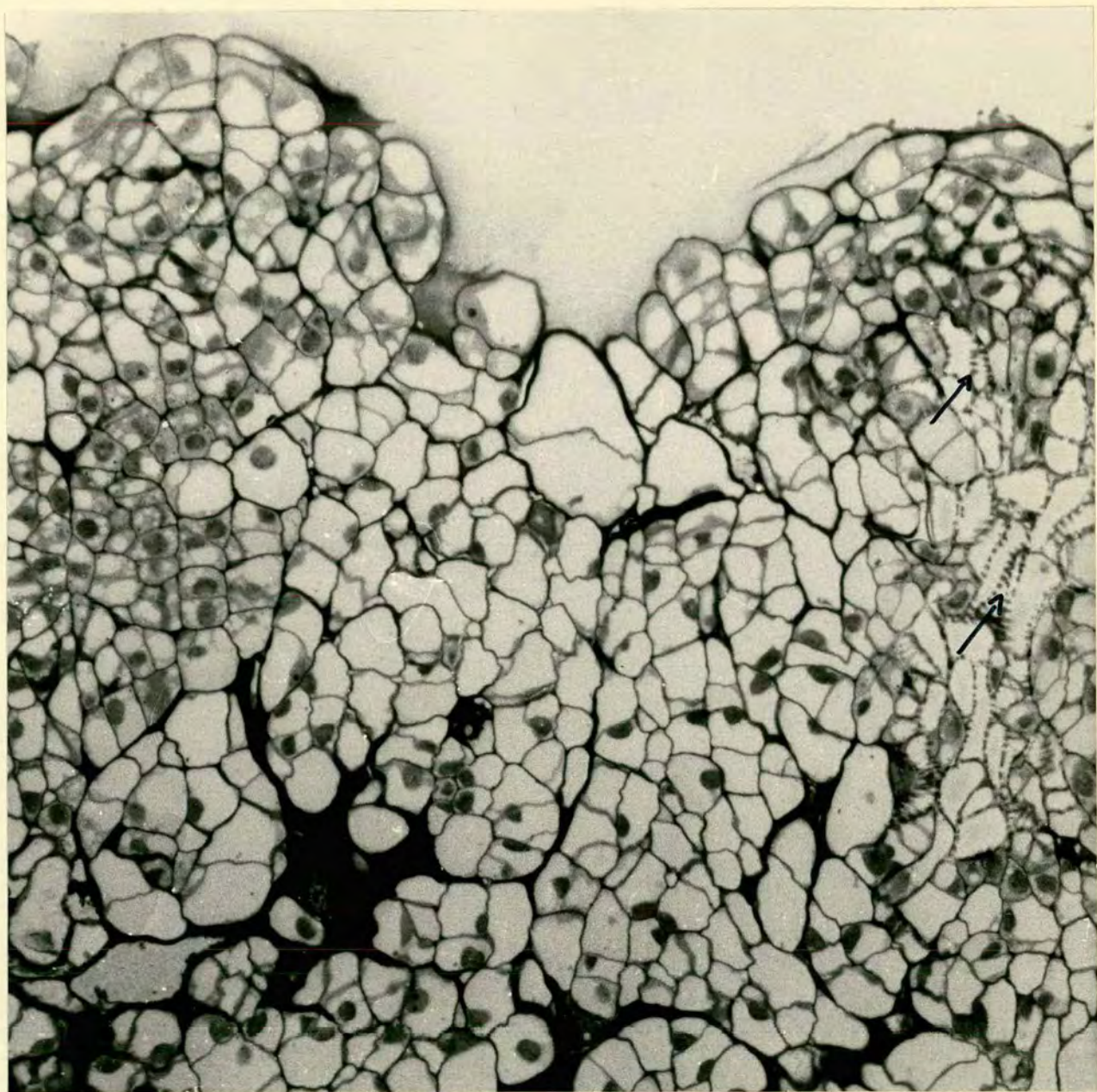


PLATE 56.

Light micrograph of outer layers of tissue cultured for 17 days (standard medium: Agar: 1). The reduction in cell size can be seen when this plate is compared with Plate 1A (uncultured). Differentiation of tracheids is shown at the right of the plate (arrowed). Fixed in acrolein, embedded in HPMA and stained with acid fuchsin and toluidine blue. X 580.

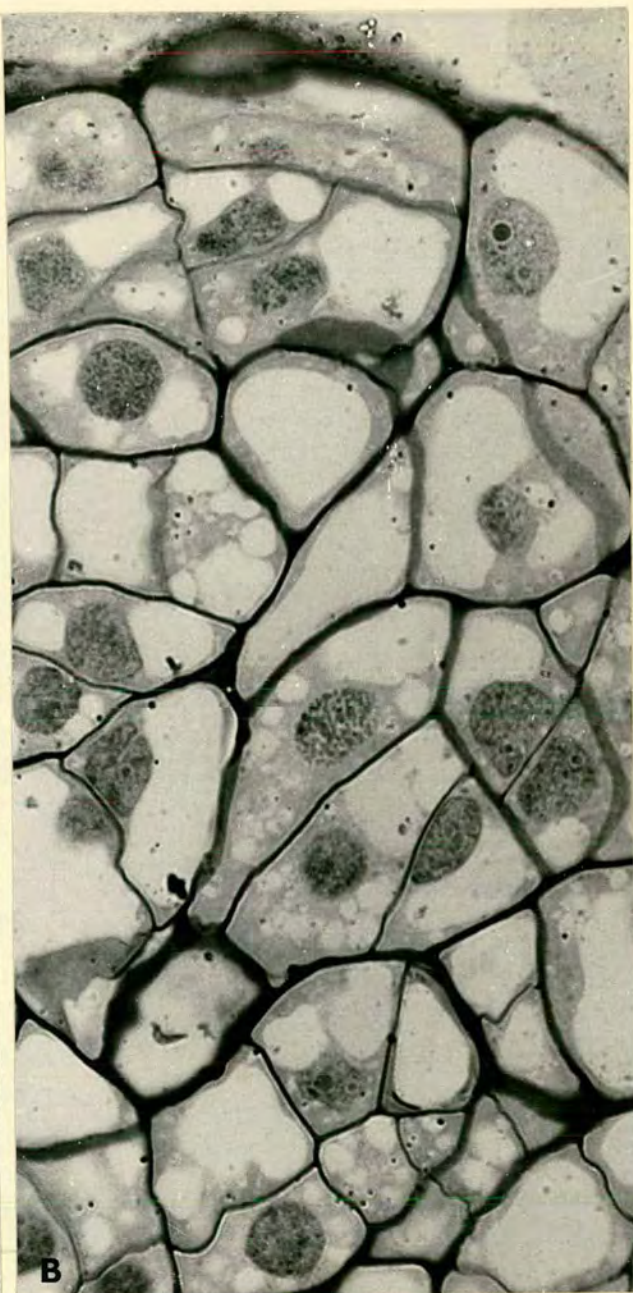
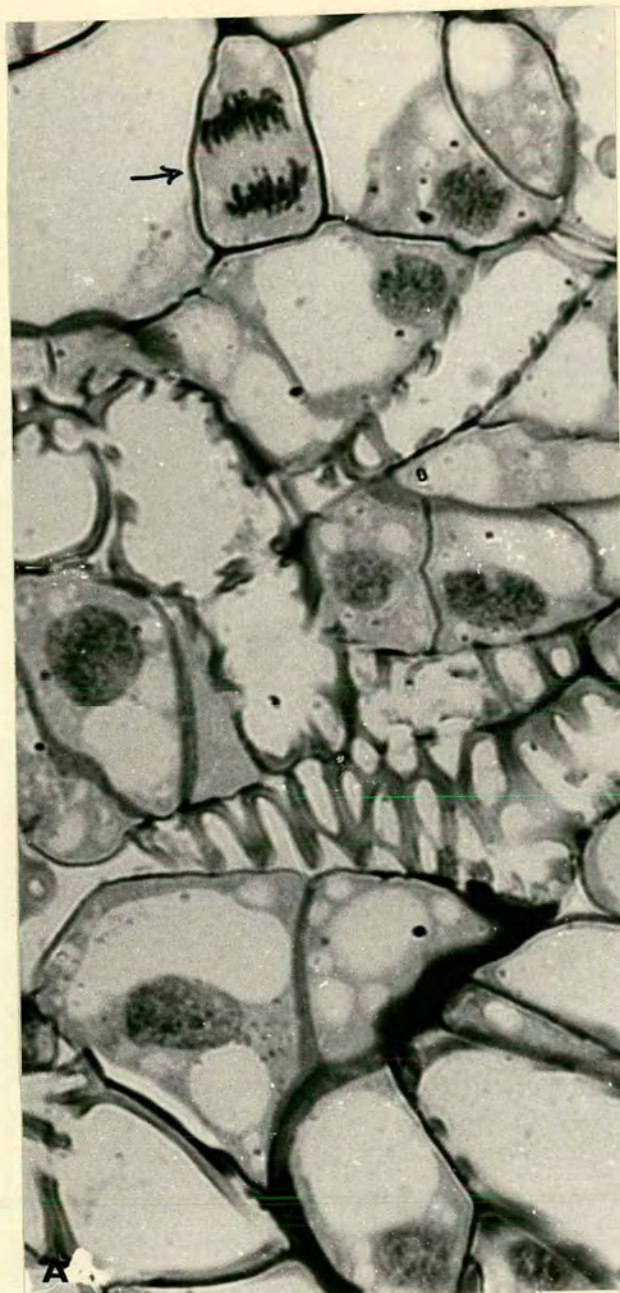


PLATE 57.

Light micrographs of the same tissue as in Plate 56. Cultured 17 days (standard medium: Agar: 1). Fixed in acrolein, embedded in HPMA and stained with acid fuchsin and toluidine blue.

A An area where tracheids are differentiating is shown. One cell is in anaphase (arrowed). X 2,300.

B Meristematic callus cells near the outside (upper portion) of the explant (Cf. Plate 1B: uncultured material). X 1,800.



PLATE 58.

Electron micrograph of tissue cultured 6 days (standard medium: Liquid: 2) showing the degenerating outer cells collapsing to form a rind on the surface of the explant. Meristematic callus cells lie beneath this. (G,Os.U,Pb) X 4,000.

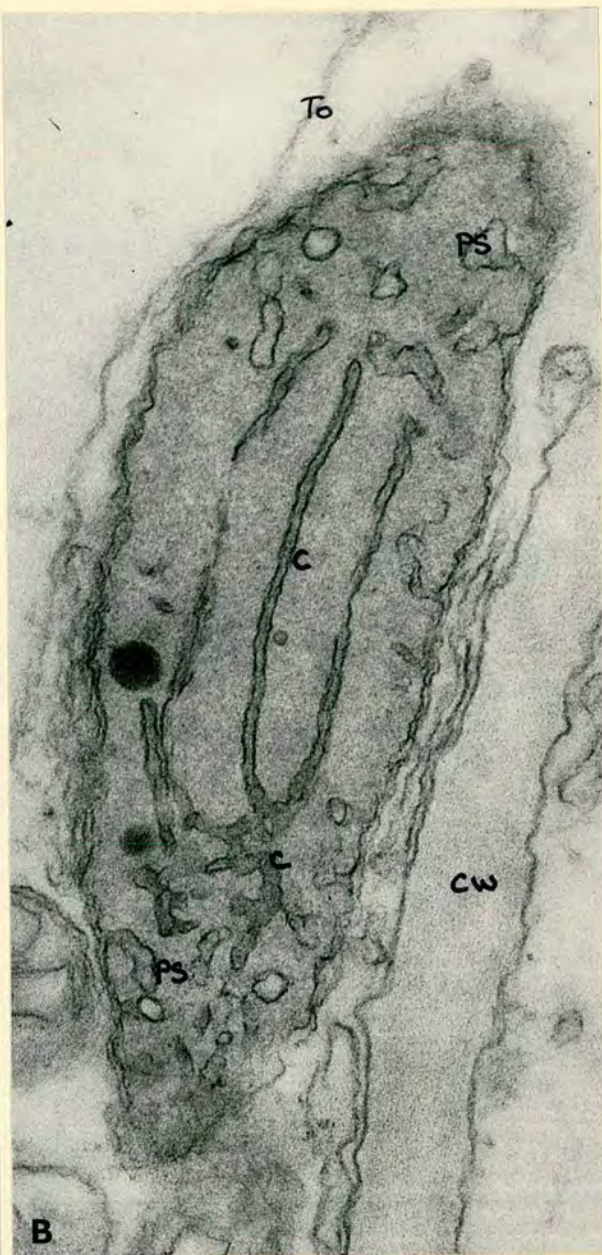
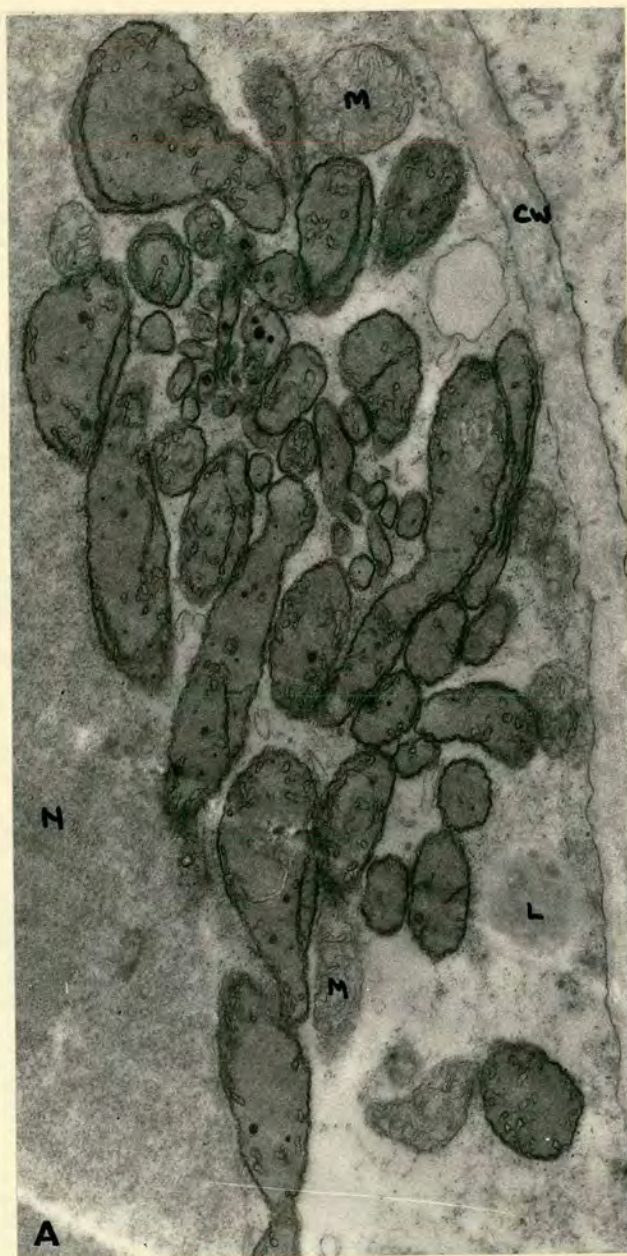


PLATE 59.

Plastids in cultured tissue (G, OS.Pb)

A A cluster of plastids in tissue cultured 6 days (standard medium: Liquid: 2) X 20,000

B Tissue cultured 28 days in light (standard medium: Agar: 1). A plastid containing lamellae as part of the central system is shown. There are no grana. X 72,000.

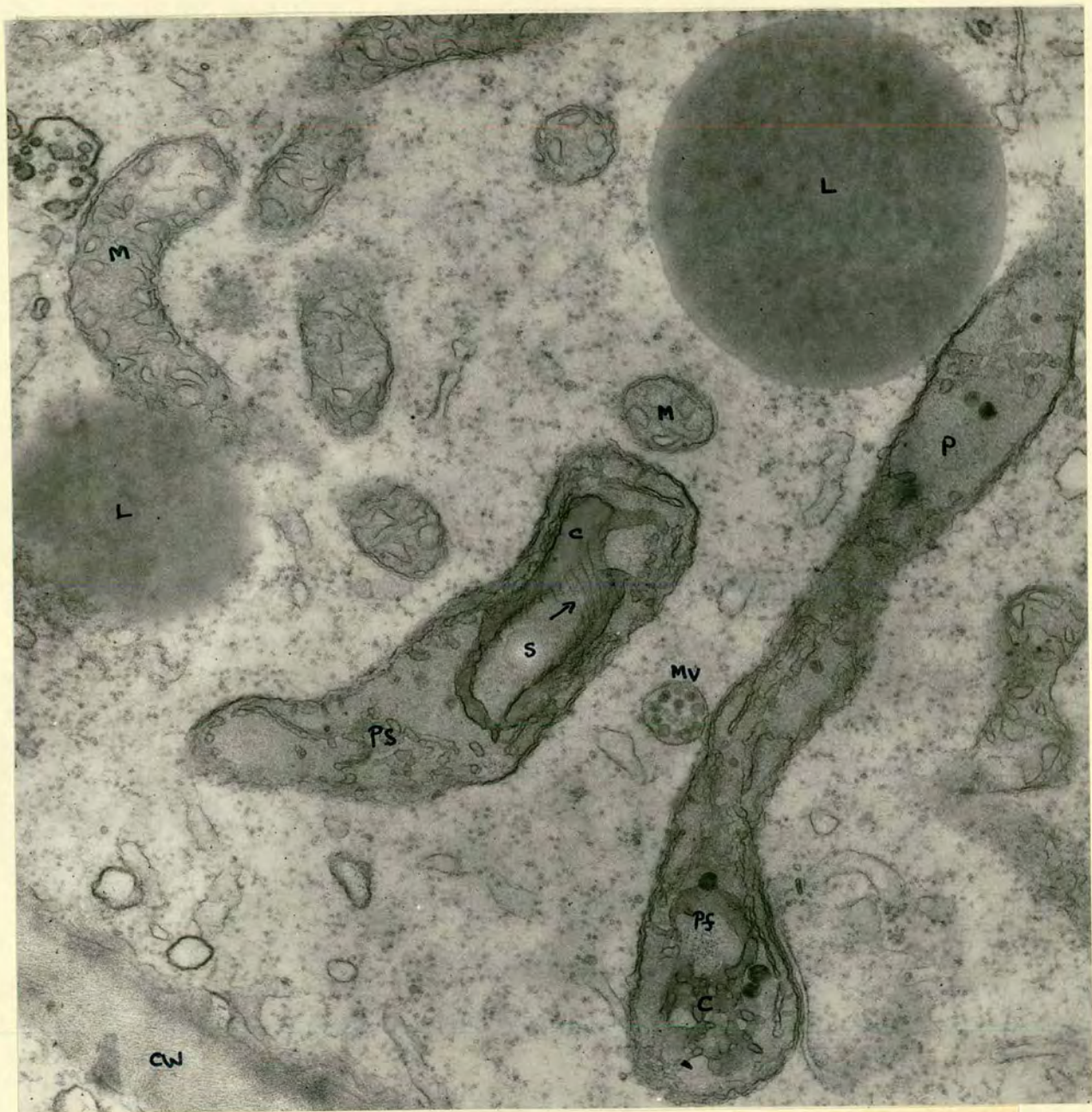


PLATE 60.

Tissue cultured 22 days (standard medium: Liquid: 2). 'Ribs' (arrowed) are associated with the central system in one of the plastids. The plastids also contain starch and phytoferritin. Large lipid bodies are shown. (G, Os.U,Pb) X 36,000.



PLATE 61.

Plastids in tissue cultured 22 days (standard medium: Liquid: 2).

'Ribs' line the inner surface of loops of the central system.

(G, Os, Pb)

A X 43,000.

B X 58,000.

C Ribs with an electron transparent core and smaller ones (arrowed) which appear solid are shown. X 140,000.

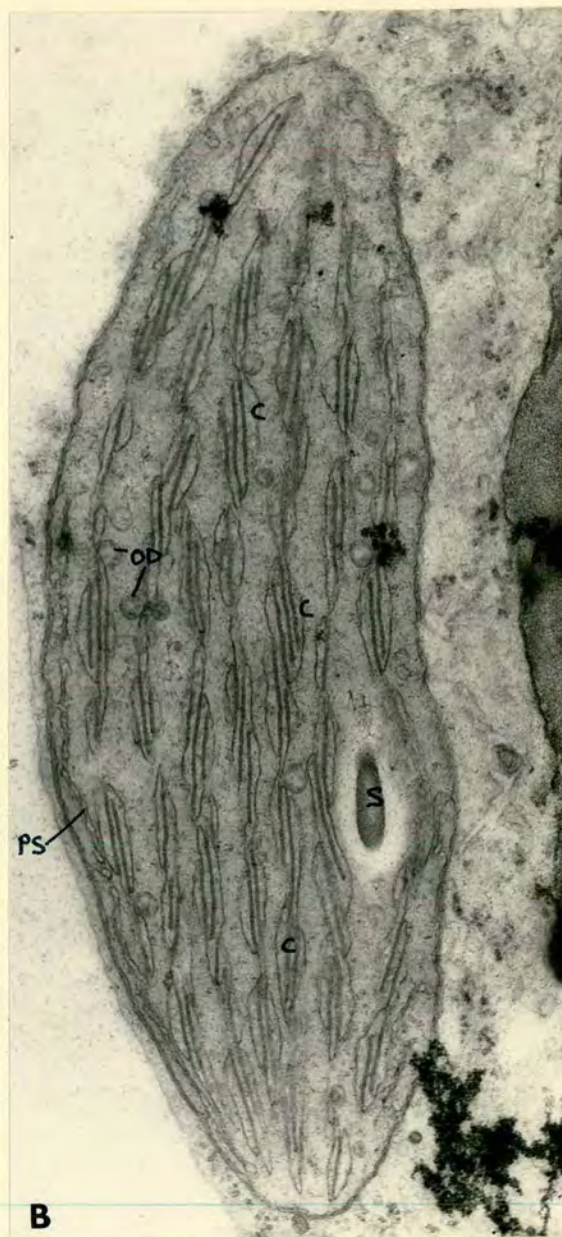
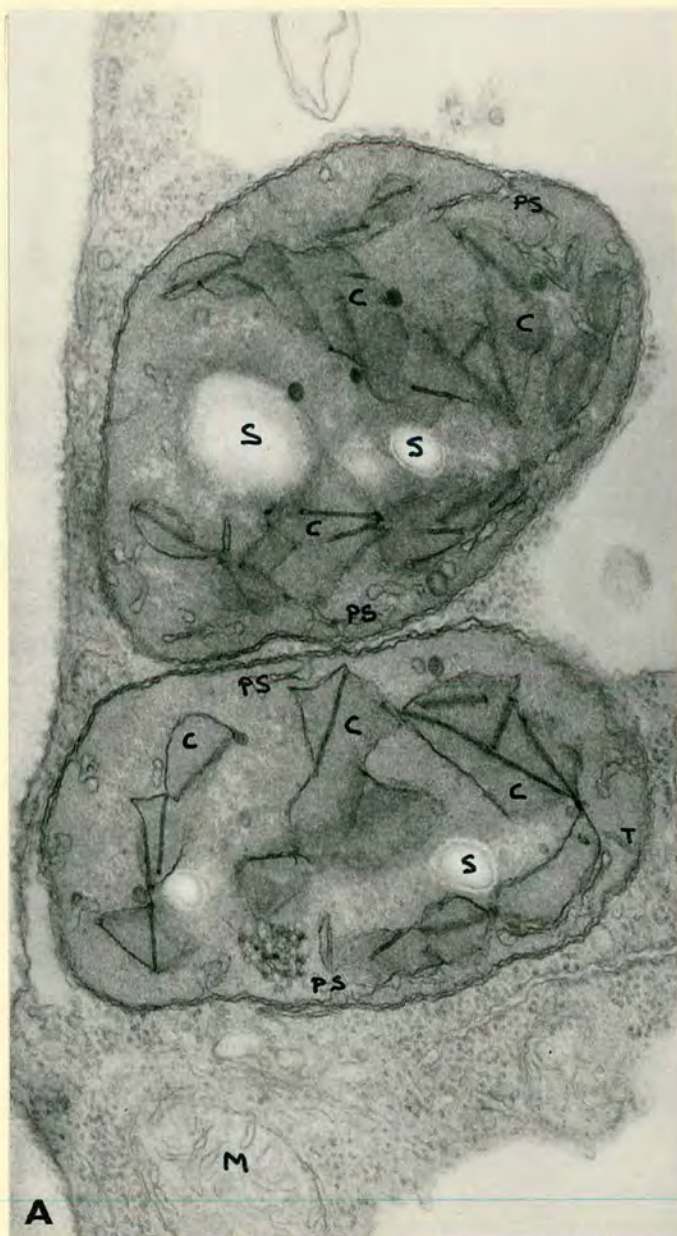


PLATE 62.

Plastids in tissue cultured in the light for 5 weeks. (No CM: Agar:1).
(G, Os, Pb).

- A The central system of each plastid is partially fragmented by the invagination of the bounding membrane. Distinct peripheral and tubular systems are shown. X 43,000.
- B Chloroplast and thylakoid system composed of grana and stroma lamellae. A peripheral system, starch and osmiophilic droplets are also shown. X 35,000.

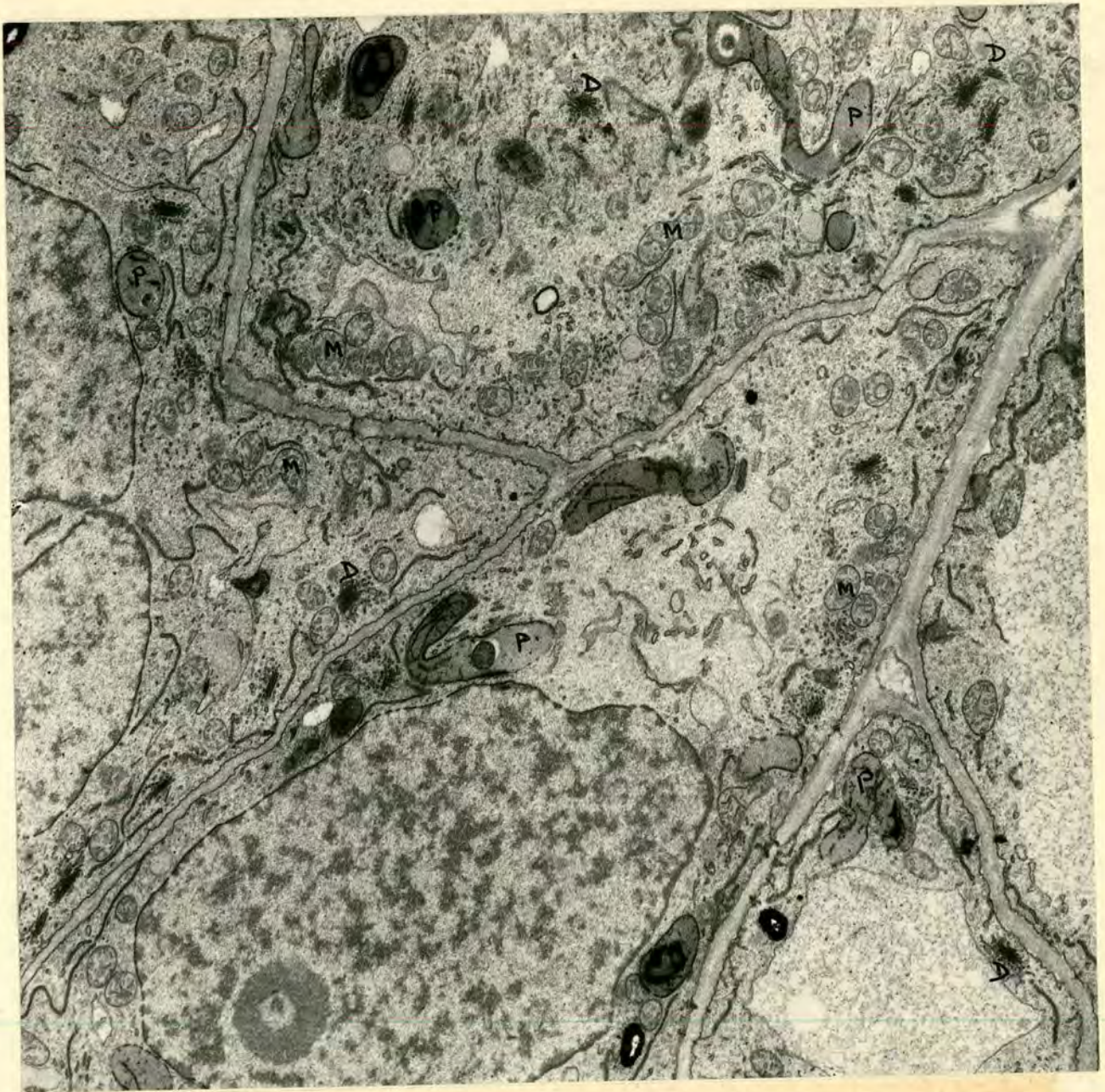


PLATE 63.

Tissue cultured 6 days (standard medium: Liquid: 2).

Small circular and elongate mitochondrial profiles are shown. The dictyosomes and plastids are also abundant. (G,Mn,Pb) X 7,500.

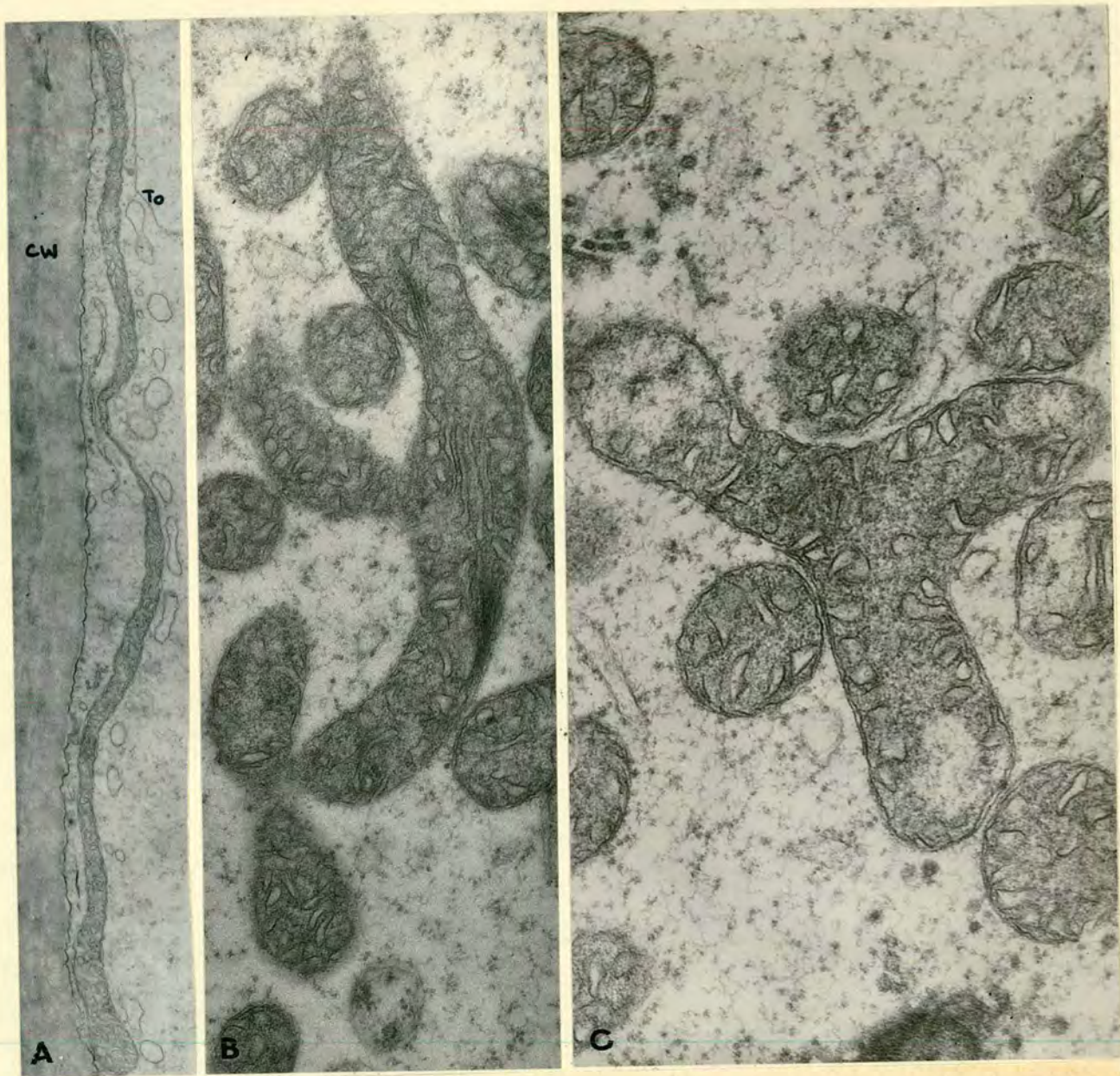


PLATE 64.

Mitochondria in tissue cultured 22 days (standard medium: Liquid: 2).

A An elongate mitochondrial profile (G,Os,Pb) X 16,000.

B&C Branching mitochondria (G,Os,Ba(MnO₄)₂) B X 31,000. C X 51,000.

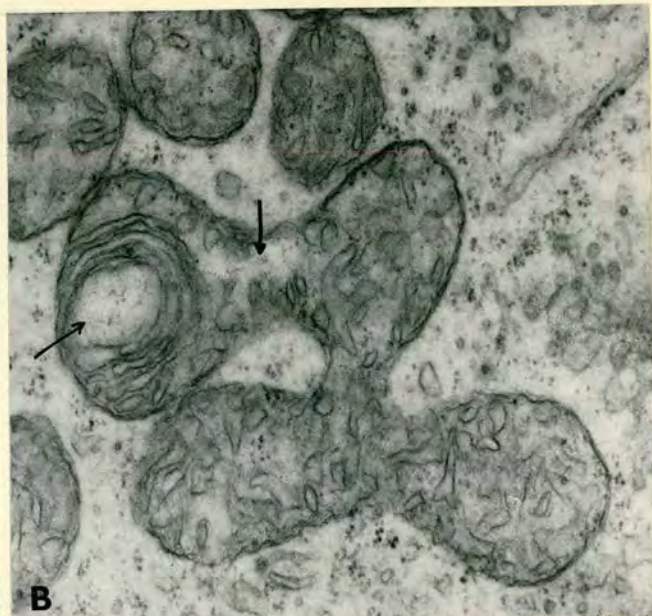


PLATE 65.

Mitochondria in cultured tissue (standard medium: Liquid: 2)
(G, Os, Pb).

A Cultured 22 days. Extended cristae lie along the length of the mitochondrion. These cristae are associated with fine fibrils (arrowed).
X 74,000.

B&C Mitochondria showing cristae forming circles. Fine fibrils are arrowed in B. B cultured 6 days X 36,000. C cultured 22 days
X 68,000.

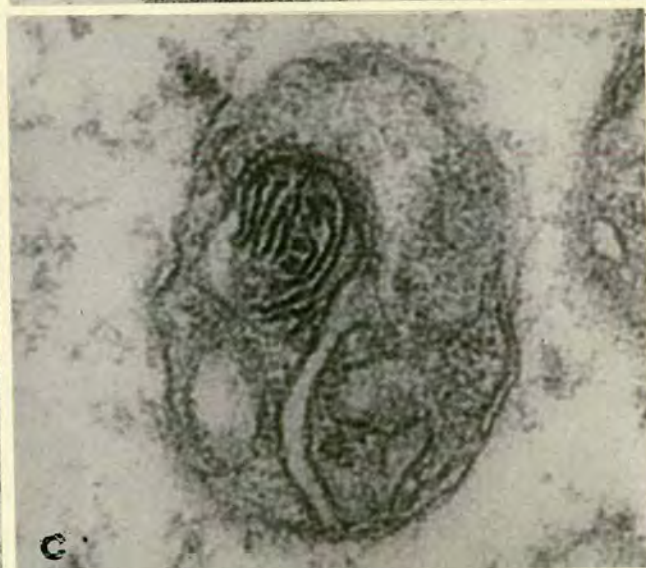


PLATE 66.

Mitochondria in tissue cultured for 22 days (standard medium: Liquid: 2) showing folded lamellae in the matrix. (G, Os. Ba(MnO₄)₂).

A&B Longitudinally sectioned lamellae.

A X 26,000. B (enlargement of A) X 68,000.

C Transversely sectioned lamellae x148,000.

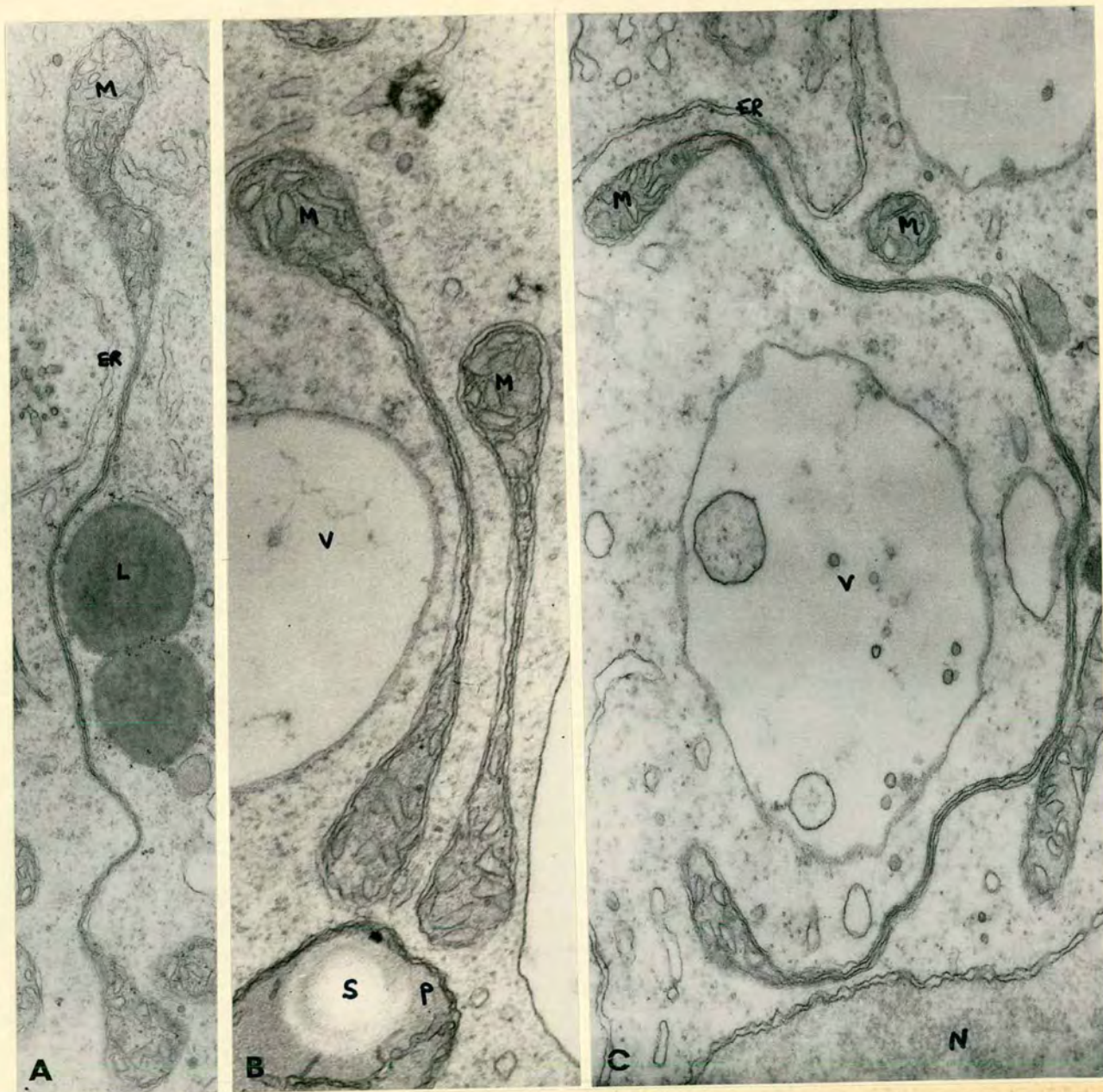


PLATE 67.

Mitochondria in tissue cultured for 28 days (Standard medium: Liquid: 2).
(G,Os,Pb).

A&B Sections through mitochondrial plates with the normal mitochondrial structure, including cristae, around the rim but no cristae within the envelope in the centre of the plate. A X 23,000. B X 43,000.

C Section through bell shaped mitochondrion. As in A & B the rim is composed of the normal mitochondrial structure but there are no cristae within the envelope in the dome of the bell. Note the vacuole within the bell X 32,000.

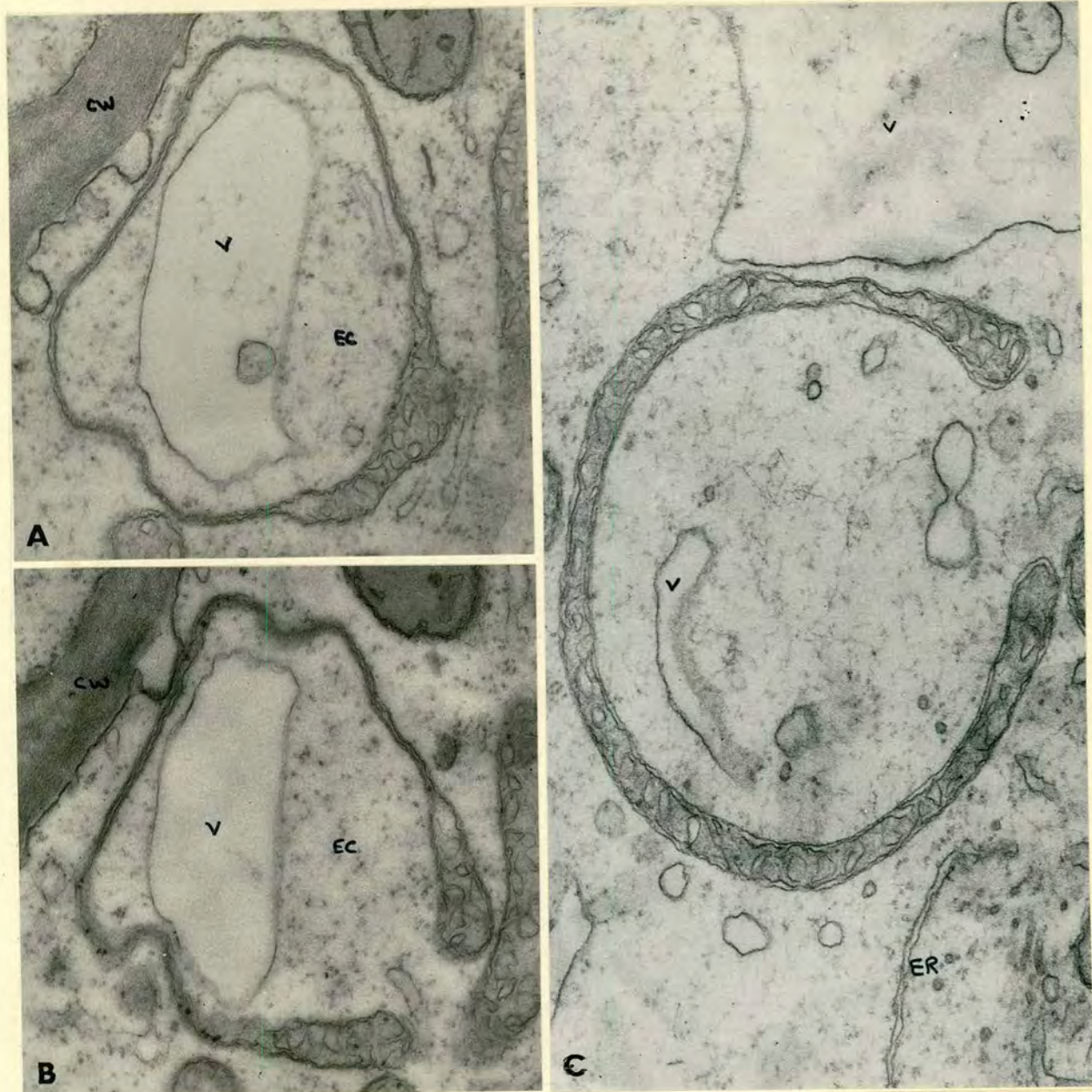


PLATE 68.

Mitochondria in cultured tissue (G,Os.Pb).

A&B Cultured 22 days (Standard medium: Liquid: 2). Sections of the same mitochondrion separated by 800 Å gap. The encircled cytoplasm in A communicates with the surrounding cytoplasm in B. Note the vacuole in the encircled cytoplasm X 33,000.

C Cultured 28 days in light (standard medium: Agar: 1). A bangle shaped profile which could represent a section cut through a bell shaped mitochondrion. (See a_1 - a_2 on Fig. 4). X 42,000.

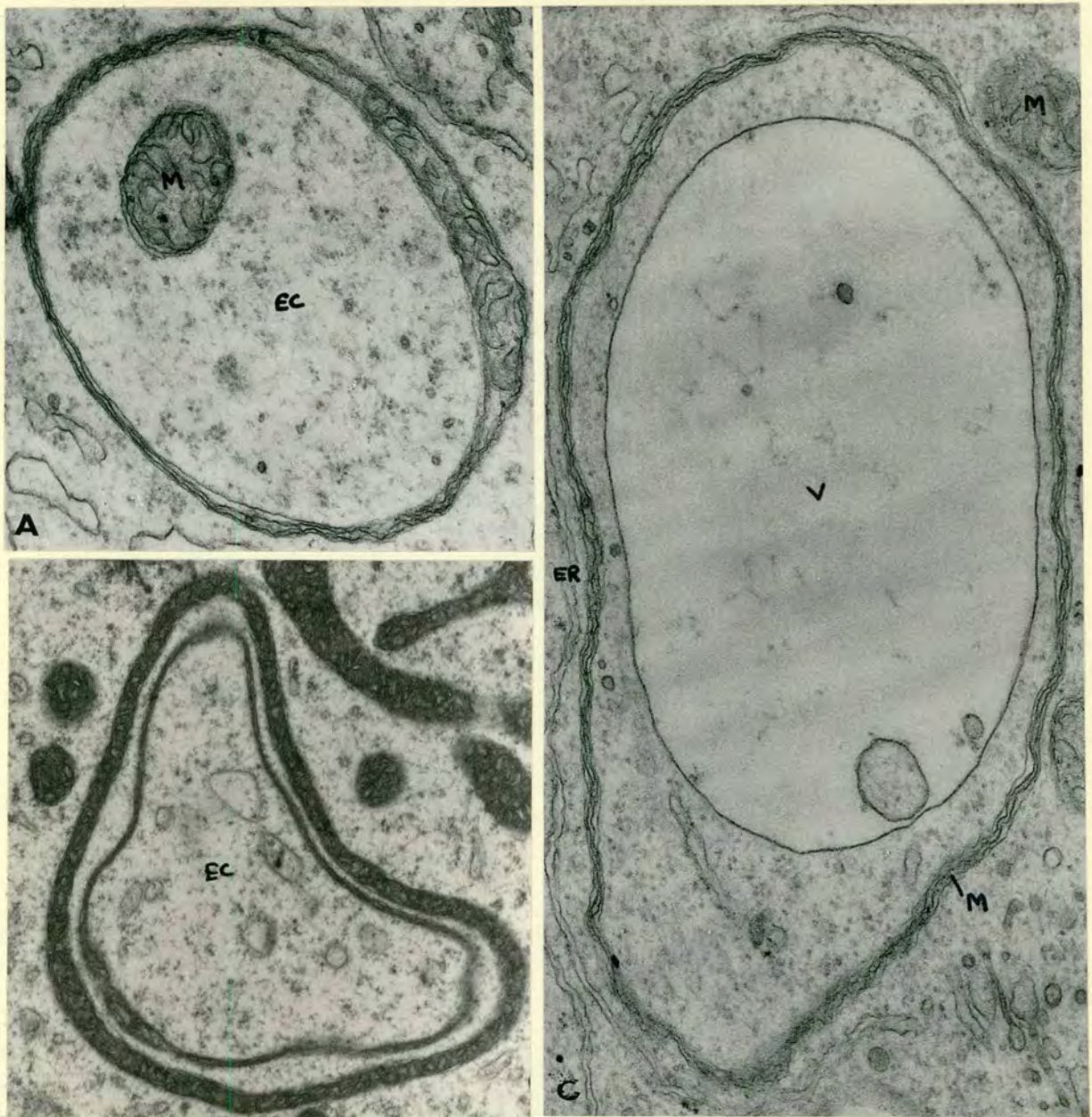


PLATE 69.

Ring shaped mitochondrial profiles in cultured tissue. They could represent sections cut through a bell shaped mitochondrion.

A Cultured 28 days in light (standard medium: Agar: 1).

Section $c_1 - c_2$ in Fig 4. (G,Os.Pb) X 43,000.

B Cultured 22 days (standard medium: Liquid: 2). One bell fitting over another is shown. The outer bell may be section $b_1 - b_2$ and the inner bell $d_1 - d_2$ on Fig 4. (G,Os. Ba(MnO₄)₂). X 20,000

C Cultured 28 days in light (standard medium: Agar: 1). The mitochondrial ring (section $e_1 - e_2$ on Fig 4) contains no cristae. Note the large vacuole within the ring. (Enlargement of Plate 70) (G,Os.Pb). X 39,000.

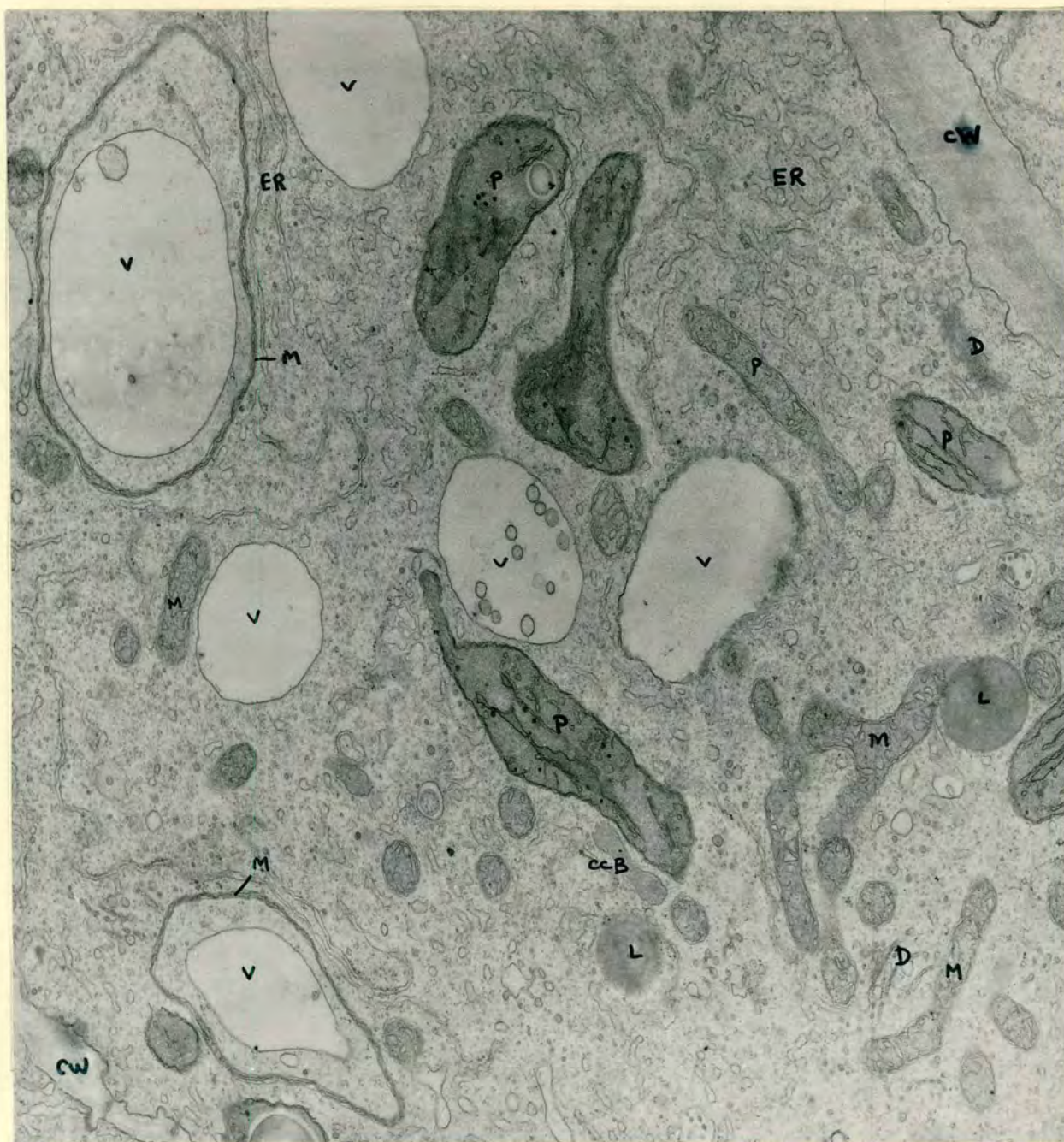


PLATE 70.

Part of a cell from tissue cultured for 28 days in light.
 (standard medium: Agar: 1). Sections of both branched and bell
 shaped mitochondria (See Plate 69) are shown. Note the vacuoles
 within the bell shaped ones. No grana have developed in the plastids.
 Smooth and rough ER are abundant (G,Os,Pb) X 16,000.

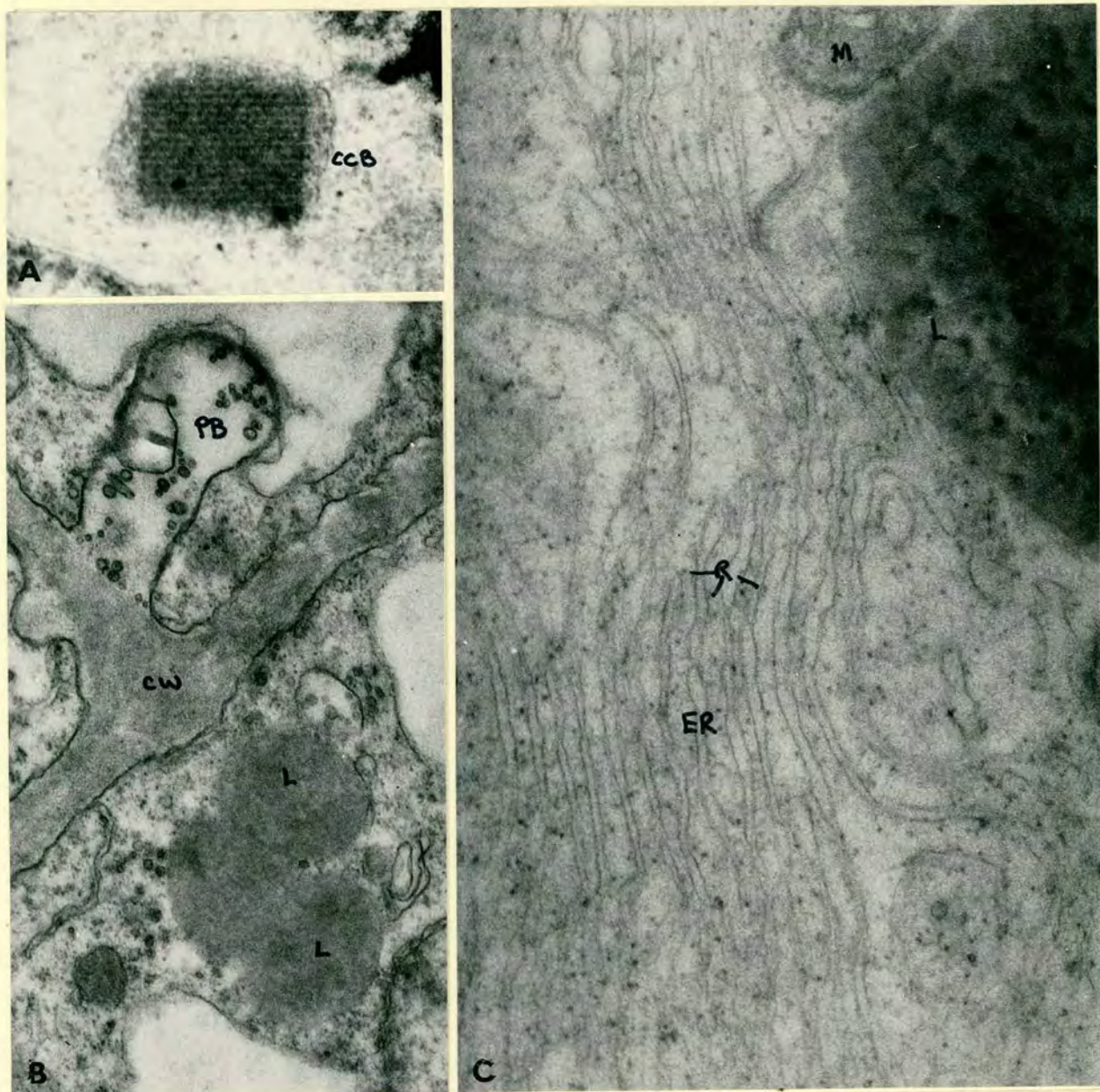


PLATE 71.

Cultured tissue.

- A Cultured 28 days (standard medium: Liquid: 2). A crystal containing body with a large crystal. (G,Os.U,Pb) X 103,000.
- B Cultured 10 days in the light (standard medium: Agar: 1). A cluster of 3 lipid bodies and a paramural body are shown (G,Os.Pb) X 31,000.
- C Cultured 28 days (standard medium: Liquid: 2). Only a few ribosomes are bound to the ER. Part of a lipid body is also shown (G,Os.U,Pb) X 64,000.

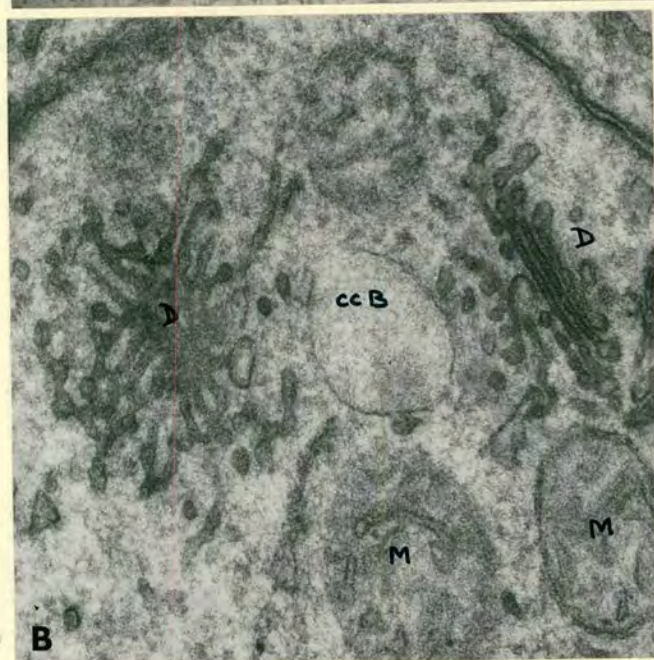


PLATE 72.

Developing vacuoles, and dictyosomes in cultured tissue.

A Cultured 28 days in light (Standard medium: Agar: 1). Small vacuoles are connected to membranes similar to ER. (G,Os,Pb)
X 33,000.

B Cultured 4 days (standard medium: Liquid: 2). Dictyosomes consist of flat cisternae and many vesicles. The small crystal in the CCB is not well preserved with this fixation procedure. (G,Mn,Pb).
X 43,000.

C Cultured 28 days (standard medium: Liquid: 2). A dictyosome with curved cisternae and few vesicles. (G, Os,U,Pb). X 65,000.